

**AMENDMENTS TO THE CLAIMS**

Please replace all listings of the claims with the following:

1. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:
  - a) providing:
    - (i) at least one RNA target;
    - (ii) at least one primer or nucleic acid construct comprising sequences complementary to a sequence in said RNA target;
    - (iii) modifying reagents for the modification of the 3' end of said RNA target;  
and
    - (iii) synthesizing reagents for the synthesis of a first nucleic acid copy;
  - b) modifying the 3' end of said RNA target by said modifying reagents such that the 3' end becomes non-functional;
  - c) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
  - d) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.
  
2. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:
  - a) providing:
    - (i) at least one RNA target;
    - (ii) at least one primer or nucleic acid construct comprising sequences complementary to a sequence in said RNA target;
    - (iii) modifying reagents for the modification of the 3' end of said RNA target;  
and
    - (iv) synthesizing reagents for the synthesis of a first nucleic acid copy;

- b) modifying the 3' end of said RNA target by said modifying reagents such that the hydroxyl group at the 3' end of said RNA target is removed or blocked;
  - c) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
  - d) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.
3. (Original) The method of claim 1 or 2 wherein said synthesizing reagents comprise a DNA polymerase with reverse transcriptase activity.
4. (Original) The method of claim 2 wherein said modifying reagents comprise chemicals for chemical reactions or enzymes for enzymatic reactions.
5. (Original) The method of claim 4 wherein said chemicals comprise reagents for carrying out periodate oxidation of the 3' end of said RNA target.
6. (Original) The method of claim 4 wherein said enzymes comprise poly A polymerase or T4 RNA ligase.
7. (Original) The method of claim 4, wherein said enzymatic reactions comprise ligation of a moiety wherein:
- a) said moiety comprises one or more nucleotides or nucleotide analogues;
  - b) said moiety comprises a 5' end capable of being ligated to the 3' end of a nucleic acid; and
  - c) said moiety lacks an extendable 3' OH group.

8. (Original) The method of claim 2 wherein said primer or nucleic acid construct comprises a sequence complementary to an inherent UDT.
9. (Original) The method of claim 8 wherein said inherent UDT comprises 3' poly A segments or consensus segments.
10. (Original) The method of claim 9 wherein said consensus segments comprise signal sites for poly A addition, splicing elements, and multicopy repeats.
11. (Original) The method of claim 2 further comprising the steps of:
  - a) providing additional synthesizing reagents for the synthesis of a complementary copy of said first nucleic acid copy;
  - b) separating said RNA target from said first nucleic acid copy or degrading said RNA target; and
  - c) synthesizing said complementary copy.
12. (Original) The method of claim 11 wherein said additional synthesizing reagents comprise a DNA polymerase.
13. (Original) The method of claim 11 wherein said additional synthesizing reagents comprise a DNA polymerase containing RNase H activity.
14. (Original) The method of claim 11 wherein said additional synthesizing reagents comprise a DNA polymerase and RNase H.
15. (Original) The method of claim 11 wherein said additional synthesizing reagents comprise:
  - a) enzymes for the addition of a non-inherent UDT to said first nucleic acid copy;
  - b) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT; and
  - c) a DNA polymerase.

16. (Original) The method of claim 15 wherein said addition takes place by ligation of a nucleic acid sequence comprising a UDT.
17. (Original) The method of claim 15 wherein said addition takes place by the action of Terminal Deoxynucleotidyl Transferase.
18. (Original) The method of claim 11 wherein said primer comprises an RNA promoter sequence.
19. (Original) The method of claim 15 wherein said reverse primer or reverse nucleic acid construct comprises an RNA promoter sequence.
20. (Original) The method of claim 18 or 19 further comprising the steps of:
  - a) providing reagents for RNA transcription; and
  - b) carrying out said RNA transcription.
21. (Original) The method of claim 18 or 19 further comprising the steps of:
  - a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs and NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
22. (Original) The method of claim 15 wherein said primer or nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.
23. (Original) The method of claim 22 further comprising the steps of:
  - a) providing:
    - (i) additional synthesizing reagents; and
    - (ii) RNase H;

- b) degrading all or a portion of the RNA segment of said extended ribonucleotide primer or ribonucleotide nucleic acid construct;
- c) annealing a second copy of said ribonucleotide primer or ribonucleotide nucleic acid construct to said complementary copy; and
- d) extending said ribonucleotide primer or ribonucleotide nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

24. (Original) The method of claim 15 wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

25. (Original) The method of claim 23 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;
- c) annealing a second copy of said chimeric primer or chimeric nucleic acid construct to said complementary copy; and
- d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

26. (Original) The method of claim 15 wherein said reverse primer or reverse nucleic acid construct is a reverse ribonucleotide primer or reverse ribonucleotide nucleic acid construct.

27. (Original) The method of claim 26 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;

- b) degrading all or a portion of the RNA segment of said extended reverse primer or said reverse nucleic acid construct;
- c) annealing a second copy of said reverse primer or said reverse nucleic acid construct to said complementary copy; and
- d) extending said reverse primer or said reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

28. (Original) The method of claim 27 wherein said primer or nucleic acid construct is also a ribonucleotide primer or ribonucleotide nucleic acid construct.

29. (Original) The method of claim 15 wherein said reverse primer is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides

30. (Original) The method of claim 29 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse chimeric primer or reverse chimeric nucleic acid construct;
- c) annealing a second copy of said reverse chimeric primer or reverse chimeric nucleic acid to said complementary copy; and
- d) extending said reverse chimeric primer or reverse chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

31. (Original) The method of claim 30 wherein said primer or nucleic acid construct is also a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

32. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:

- a) providing:
    - (i) at least one RNA target;
    - (ii) at least one primer or nucleic acid construct comprising a sequence complementary to a sequence in said RNA target;
    - (iii) at least one ribonucleotide analogue lacking a 3' OH group;
    - (iv) modifying reagents for the addition of said ribonucleotide analogue; and
    - (v) synthesizing reagents for the synthesis of a first nucleic acid copy;
  - b) modifying said RNA target by said modifying reagents such that said ribonucleotide analogue is added to the 3' end of said RNA target;
  - c) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
  - d) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.
33. (Original) The method of claim 32 wherein said synthesizing reagents comprise a DNA polymerase with reverse transcriptase activity.
34. (Original) The method of claim 32 wherein said modifying reagents comprise an enzyme which adds said ribonucleotide analogue to the 3' end of said RNA target.
35. (Original) The method of claim 34 wherein said enzyme is poly A polymerase.
36. (Original) The method of claim 34 wherein said enzyme is T4 RNA ligase.
37. (Original) The method of claim 32 wherein said ribonucleotide analogue comprises cordycepin triphosphate or 3' aminoadenosine.

38. (Original) The method of claim 37 wherein said modifying reagents comprise an enzyme capable of adding said ribonucleotide analogue.
39. (Original) The method of claim 38 wherein said enzyme is poly A polymerase.
40. (Original) The method of claim 38 wherein said enzyme is T4 RNA ligase.
41. (Original) The method of claim 32 further comprising the steps of:
- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said first nucleic acid copy;
  - b) separating said RNA target from said first nucleic acid copy or degrading said RNA target; and
  - c) synthesizing said complementary copy.
42. (Original) The method of claim 41 wherein said additional synthesizing reagents comprise DNA polymerase.
43. (Original) The method of claim 41 wherein said additional synthesizing reagents comprise DNA polymerase containing RNase H activity.
44. (Original) The method of claim 41 wherein said additional synthesizing reagents comprise DNA polymerase and RNase H.
45. (Original) The method of claim 41 wherein said additional synthesizing reagents comprise:
- a) enzymes for the addition of a non-inherent UDT to said first nucleic acid copy;
  - b) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT; and
  - c) a DNA polymerase.



46. (Original) The method of claim 45 wherein said primer or nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.

47. (Original) The method of claim 46 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended ribonucleotide primer or ribonucleotide nucleic acid construct;
- c) annealing a second copy of said ribonucleotide primer or ribonucleotide nucleic acid construct to said complementary copy; and
- d) extending said ribonucleotide primer or ribonucleotide nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

48. (Original) The method of claim 45 wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

49. (Original) The method of claim 48 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;
- c) annealing a second copy of said chimeric primer or chimeric nucleic acid construct to said complementary copy; and
- d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

50. (Original) The method of claim 45 wherein said reverse primer or reverse nucleic acid construct is a reverse ribonucleotide primer or reverse ribonucleotide nucleic acid construct.

51. (Original) The method of claim 50 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse primer or said reverse nucleic acid construct;
- c) annealing a second copy of said reverse primer or said reverse nucleic acid construct to said complementary copy; and
- d) extending said reverse primer or said reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

52. (Original) The method of claim 51 wherein said primer or nucleic acid construct is also a ribonucleotide primer or ribonucleotide nucleic acid construct.

53. (Original) The method of claim 45 wherein said reverse primer or reverse nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

54. (Original) The method of claim 53 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse chimeric primer or reverse chimeric nucleic acid construct;
- c) annealing a second copy of said reverse chimeric primer or reverse chimeric nucleic acid construct to said complementary copy; and

d) extending said reverse chimeric primer or reverse chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

55. (Original) The method of claim 54 wherein said primer or nucleic acid construct is also a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

56. (Original) The method of claim 45 wherein said addition takes place by ligation of a nucleic acid sequence comprising a UDT.

57. (Original) The method of claim 45 wherein said addition takes place by the action of Terminal Deoxynucleotidyl Transferase.

58. (Original) The method of claim 41 wherein said primer or nucleic acid construct comprises an RNA promoter sequence.

59. (Original) The method of claim 45 wherein said reverse primer or reverse nucleic acid construct comprises an RNA promoter sequence.

60. (Original) The method of claim 58 or 59 further comprising the steps of:

- a) providing reagents for RNA transcription; and
- b) carrying out said RNA transcription.

61. (Original) The method of claim 58 or 59 further comprising the steps of:

- a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
- b) providing dNTPs or a mixture of dNTPs and NTPs; and
- c) creating a DNA transcript or a DNA/RNA chimeric transcript.

62. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:

- a) providing:
  - (i) at least one RNA target;
  - (ii) at least one non-inherent UDT wherein said UDT comprises a nucleic acid oligonucleotide that comprises a nucleotide analogue lacking a 3' OH group at the 3' terminus;
  - (iii) at least one primer or nucleic acid construct comprising sequences complementary to said non-inherent UDT;
  - (iv) addition reagents for the addition of said UDT; and
  - (vi) synthesizing reagents for the synthesis of a first nucleic acid copy;
- b) modifying said RNA by the addition of said UDT at the 3' end of said RNA target using said addition reagents;
- c) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
- d) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.

63. (Original) The method of claim 62 wherein said synthesizing reagents comprise a DNA polymerase with reverse transcriptase activity.

64. (Original) A method according to claim 62 wherein said addition reagents comprise T4 RNA ligase.

65. (Original) A method according to claim 62 wherein said nucleotide analogue comprises cordycepin, a 3' amino-ribonucleotide, a 3' amino-2' deoxyribonucleotide, a 3' amino-nucleotide analogue, a 2, 3 dideoxyribonucleotide or an acyclonucleotide terminator.

66. (Original) The method of claim 62 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said first nucleic acid copy;
- b) separating said RNA target from said first nucleic acid copy or degrading said RNA target; and
- c) synthesizing said complementary copy.

67. (Original) The method of claim 66 wherein said additional synthesizing reagents comprise DNA polymerase.

68. (Original) The method of claim 66 wherein said additional synthesizing reagents comprise DNA polymerase containing RNase H activity.

69. (Original) The method of claim 66 wherein said additional synthesizing reagents comprise DNA polymerase and RNase H.

70. (Original) The method of claim 63 wherein said additional synthesizing reagents comprise:

- a) enzymes for the addition of a non-inherent UDT to said first cDNA copy;
- b) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT; and
- c) a DNA polymerase.

71. (Original) The method of claim 70 wherein said primer or nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.

72. (Original) The method of claim 71 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents, and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended ribonucleotide primer or ribonucleotide nucleic acid construct;

- c) annealing a second copy of said ribonucleotide primer or ribonucleotide nucleic acid to said complementary copy; and
- d) extending said ribonucleotide primer or ribonucleotide nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

73. (Original) The method of claim 70 wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

74. (Original) The method of claim 73 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents, and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;
- c) annealing a second copy of said chimeric primer or chimeric nucleic acid to said complementary copy; and
- d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

75. (Original) The method of claim 70 wherein said reverse primer or reverse nucleic acid construct is a reverse ribonucleotide primer or reverse ribonucleotide nucleic acid construct.

76. (Original) The method of claim 75 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse primer or said reverse nucleic acid construct;

- c) annealing a second copy of said reverse primer or said reverse nucleic acid construct to said complementary copy; and
- d) extending said reverse primer or said reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

77. (Original) The method of claim 76 wherein said primer or nucleic acid construct is also a ribonucleotide primer or ribonucleotide nucleic acid construct.

78. (Original) The method of claim 70 wherein said reverse primer is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

79. (Original) The method of claim 78 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse chimeric primer or reverse chimeric nucleic acid construct;
- c) annealing a second copy of said reverse chimeric primer or reverse chimeric nucleic acid to said complementary copy; and
- d) extending said reverse chimeric primer or reverse chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

80. (Original) The method of claim 79 wherein said primer or nucleic acid construct is also a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

81. (Original) The method of claim 70 wherein said addition takes place by ligation of a nucleic acid sequence comprising a UDT.

82. The method of claim 70 wherein said addition takes place by the action of Terminal Deoxynucleotidyl Transferase.
83. (Original) The method of claim 66 wherein said primer or nucleic acid construct comprises an RNA promoter sequence.
84. (Original) The method of claim 70 wherein said reverse primer or reverse nucleic acid construct comprises an RNA promoter sequence.
85. (Original) The method of claim 83 or 84 further comprising the steps of:
- a) providing reagents for RNA transcription; and
  - b) carrying out said RNA transcription
86. (Original) The method of claim 83 or 84 comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs or NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
87. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:
- a) providing:
    - (i) at least one RNA target;
    - (ii) at least one nucleotide analogue;
    - (iii) addition reagents for adding a non-inherent UDT to said RNA target;
    - (iv) at least one primer or nucleic acid construct comprising sequences complementary to said UDT;
    - (v) modifying reagents for adding said nucleotide analogue to said UDT; and
    - (vi) synthesizing reagents for the synthesis of a first cDNA copy;



- b) adding said UDT to the 3' end of said RNA target using said addition reagents;
  - c) modifying said RNA by the addition of said nucleotide analogue to said UDT at the 3' end of said RNA target using said modifying reagents;
  - d) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
  - e) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.
88. (Original) The method of claim 87 wherein said synthesizing reagents comprise a DNA polymerase with reverse transcriptase activity.
89. (Original) The method of claim 87 wherein said addition reagents comprise rATP and poly A polymerase.
90. (Original) The method of claim 87 wherein said modifying reagents comprise a mixture of ribonucleotide analogues and poly A polymerase.
91. (Original) The method of claim 87 wherein said ribonucleotide analogue comprises cordycepin or a 3'-aminoribonucleotide.
92. (Original) The method of claim 87 wherein said adding and modifying steps are carried out simultaneously.
93. (Original) The method of claim 87 wherein said addition reagents comprise at least one oligonucleotide and a ligase.
94. (Original) The method of claim 93 wherein said oligonucleotide comprises deoxyribonucleotides, ribonucleotides, or a combination of deoxyribonucleotides and ribonucleotides.

95. (Original) The method of claim 94 wherein said modifying reagents comprise Terminal Deoxynucleotidyl Transferase.
96. (Original) The method of claim 95 wherein said nucleotide analogues comprise dideoxyribonucleotide or an acyclonucleotide terminator.
97. (Original) The method of claim 94 wherein said modifying reagents comprise poly A polymerase.
98. (Original) The method of claim 97 wherein said ribonucleotide analogue comprises cordycepin or 3'-aminoribonucleotide.
99. (Original) The method of claim 87 further comprising the steps of:
- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said first nucleic acid copy;
  - b) separating said RNA target from said first nucleic acid copy or degrading said RNA target; and
  - c) synthesizing said complementary copy.
100. (Original) The method of claim 99 wherein said additional synthesizing reagents comprise DNA polymerase.
101. (Original) The method of claim 99 wherein said additional synthesizing reagents comprise DNA polymerase containing RNase H activity.
102. (Original) The method of claim 99 wherein said additional synthesizing reagents comprise DNA polymerase and RNase H.
103. (Original) The method of claim 99 wherein said additional synthesizing reagents comprise:

- a) enzymes for the addition of a non-inherent UDT to said first nucleic acid copy;
- b) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT; and
- c) a DNA polymerase.

104. (Original) The method of claim 103 wherein said primer or nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.

105. (Original) The method of claim 104 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended ribonucleotide primer or ribonucleotide nucleic acid construct;
- c) annealing a second copy of said ribonucleotide primer or ribonucleotide nucleic acid construct to said complementary copy; and
- d) extending said ribonucleotide primer or ribonucleotide nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

106. (Original) The method of claim 103 wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

107. (Original) The method of claim 106 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;
- c) annealing a second copy of said chimeric primer or chimeric nucleic acid to said complementary copy; and

d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

108. (Original) The method of claim 103 wherein said reverse primer or reverse nucleic acid construct is a reverse ribonucleotide primer or reverse ribonucleotide nucleic acid construct.

109. (Original) The method of claim 108 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse primer or said reverse nucleic acid construct;
- c) annealing a second copy of said reverse primer or said reverse nucleic acid construct to said complementary copy; and
- d) extending said reverse primer or said reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

110. (Original) The method of claim 109 wherein said primer or nucleic acid construct is also a ribonucleotide primer or ribonucleotide nucleic acid construct.

111. (Original) The method of claim 103 wherein said reverse primer is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and at least one deoxyribonucleotide.

112. (Original) The method of claim 106 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;

- c) annealing a second copy of said chimeric primer or chimeric nucleic acid construct to said complementary copy; and
- d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

113. (Original) The method of claim 112 wherein said primer or nucleic acid construct is also a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and at least one deoxyribonucleotide.

114. (Original) The method of claim 103 wherein said addition takes place by ligation of a nucleic acid sequence comprising a UDT.

115. (Original) The method of claim 103 wherein said addition takes place by the action of Terminal Deoxynucleotidyl Transferase.

116. (Original) The method of claim 99 wherein said primer or nucleic acid construct comprises an RNA promoter sequence.

117. (Original) The method of claim 104 wherein said reverse primer or reverse nucleic acid construct comprises an RNA promoter sequence.

118. (Original) The method of claim 116 or 117 comprising the steps of:

- a) providing reagents for RNA transcription; and
- b) carrying out said RNA transcription

119. (Original) The method of claim 116 or 117 comprising the steps of:

- a) providing reagents for RNA transcription, said reagents comprising Mn ++, mutated RNA polymerases or a combination thereof;
- b) providing dNTPs or a mixture of dNTPs or NTPs ; and
- c) creating a DNA transcript or a DNA/RNA chimeric transcript.

120. (Original) A method for synthesizing a nucleic acid copy of at least one RNA target comprising the steps of:

- a) providing:
  - (i) at least one RNA target
  - (ii) a mixture of at least one normal ribonucleotide and at least one ribonucleotide terminator;
  - (iii) modifying reagents for forming a non-inherent homopolymeric UDT by the addition of said ribonucleotides and said ribonucleotide terminator;
  - (iv) at least one primer or nucleic acid construct comprising sequences complementary to said UDT;
  - (v) synthesizing reagents for the synthesis of a first cDNA copy;
- b) modifying said RNA target by the addition of said ribonucleotides and said ribonucleotide terminator from said mixture to said 3' end of said RNA target using said modifying reagents to form a UDT with said ribonucleotide terminator at the 3' end;
- c) contacting said modified RNA with said primer or nucleic acid construct to form a complex between said primer or nucleic acid construct and said modified RNA; and
- d) extending said primer or nucleic acid construct using said synthesizing reagents and said modified RNA target as a template to synthesize a first nucleic acid copy of said RNA target.

121. (Original) The method of claim 115 wherein said synthesizing reagents comprise a DNA polymerase with reverse transcriptase activity.

122. (Original) The method of claim 115 wherein said modifying reagents comprise poly A polymerase.

123. (Original) The method of claim 115 wherein said normal nucleotide comprises rATP, and said ribonucleotide analogue comprises cordycepin or a 3' amino-ribonucleotide.

124. (Original) The method of claim 115 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said first nucleic acid copy;
- b) separating said RNA target from said first nucleic acid copy or degrading said RNA target; and
- c) synthesizing said complementary copy.

125. (Original) The method of claim 124 wherein said additional synthesizing reagents comprise DNA polymerase.

126. (Original) The method of claim 124 wherein said additional synthesizing reagents comprise DNA polymerase containing RNase H activity.

127. (Original) The method of claim 124 wherein said additional synthesizing reagents comprise DNA polymerase and RNase H.

128. (Original) The method of claim 124 wherein said additional synthesizing reagents comprise:

- a) enzymes for the addition of a non-inherent UDT to said first nucleic acid copy;
- b) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT; and
- c) a DNA polymerase.

129. (Original) The method of claim 128 wherein said primer or nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.

130. (Original) The method of claim 129 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;

- b) degrading all or a portion of the RNA segment of said extended ribonucleotide primer or ribonucleotide nucleic acid construct;
- c) annealing a second copy of said ribonucleotide primer or ribonucleotide nucleic acid construct to said complementary copy; and
- d) extending said ribonucleotide primer or ribonucleotide nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

131. (Original) The method of claim 128 wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

132. (Original) The method of claim 131 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended chimeric primer or chimeric nucleic acid construct;
- c) annealing a second copy of said chimeric primer or chimeric nucleic acid construct to said complementary copy; and
- d) extending said chimeric primer or chimeric nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

133. (Original) The method of claim 128 wherein said reverse primer or reverse nucleic acid construct is a ribonucleotide primer or ribonucleotide nucleic acid construct.

134. (Original) The method of claim 133 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse primer or said reverse nucleic acid construct;



- c) annealing a second copy of said reverse primer or said reverse nucleic acid construct to said complementary copy; and
- d) extending said reverse primer or said reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

135. (Original) The method of claim 134 wherein said primer or nucleic acid construct is also a ribonucleotide primer or ribonucleotide nucleic acid construct.

136. (Original) The method of claim 128 wherein said reverse primer is a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and at least one deoxyribonucleotide

137. (Original) The method of claim 136 further comprising the steps of:

- a) providing:
  - (i) additional synthesizing reagents; and
  - (ii) RNase H;
- b) degrading all or a portion of the RNA segment of said extended reverse primer or reverse nucleic acid construct;
- c) annealing a second copy of said reverse primer or reverse nucleic acid to said complementary copy; and
- d) extending said reverse primer or reverse nucleic acid construct by said additional synthesizing reagents using said complementary copy as a template.

138. (Original) The method of claim 137 wherein said primer or nucleic acid construct is also a chimeric primer or chimeric nucleic acid construct comprising an RNA segment and one or more deoxyribonucleotides.

139. (Original) The method of claim 128 wherein said addition takes place by ligation of a nucleic acid sequence comprising a UDT.

140. (Original) The method of claim 128 wherein said addition takes place by the action of Terminal Deoxynucleotidyl Transferase.

141. (Original) The method of claim 123 wherein said primer comprises an RNA promoter sequence.

142. (Original) The method of claim 128 wherein said reverse primer comprises an RNA promoter sequence.

143. (Original) The method of claim 141 or 142 comprising the steps of:

- a) providing reagents for RNA transcription; and
- b) carrying out said RNA transcription.

144. (Original) The method of claim 141 or 142 comprising the steps of:

- a) providing reagents for RNA transcription, said reagents comprising Mn<sup>++</sup>, mutated RNA polymerases or a combination thereof;
- b) providing dNTPs or a mixture of dNTPs or NTPs; and
- c) creating a DNA transcript or a DNA/RNA chimeric transcript.

145. (Original) The method of claim 11, 23, 25, 27, 30, 41, 47, 49, 51, 54, 66, 72, 74, 76, 79, 99, 105, 107, 109, 112, 123, 130, 132, 134, or 137, wherein said additional synthesizing reagents comprise E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript or any mutational variations of any of the preceding.

146. (Original) The method of claim 13, 14, 23, 25, 27, 30, 43, 44, 47, 49, 51, 54, 68, 69, 72, 74, 76, 79, 99, 101, 102, 105, 107, 109, 112, 125, 126, 130, 132, 134, or 137, wherein

multiple rounds of said degradation by said RNase H and extension by said additional synthesizing reagents are carried out.

147. (Original) The method of claim 3, 33, 63, 88, 121 wherein said synthesizing reagents comprise Bst DNA polymerase, Bca DNA polymerase, Tth DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript, any mutational variations of the preceding, or any combination of the preceding.

#### Modification of 3' DNA ends

148. (Original) A method for synthesizing a copy of at least one DNA target comprising the steps of:

(a) providing

(i) at least one DNA target

(ii) addition reagents for the addition of at least one ribonucleotide to the end of said DNA target; and

(iii) modifying reagents for the treatment of the 3' end of a ribonucleotide to inhibit or prevent its extension.

(iv) at least one primer or nucleic acid construct comprising sequences complementary to sequences in said DNA target; and

(v) synthesizing reagents for the synthesis of at least one copy of said DNA target;  
(b) modifying said DNA target by the addition of at least one ribonucleotide to said DNA target;

(c) treating said modified DNA target with said modifying reagents to render the 3' end of said modified DNA target unextendable;

(d) contacting said modified DNA target with said primer to form a complex between said primer and said modified DNA target; and

(e) extending said primer by said synthesizing reagents and using said modified DNA target as a template to synthesize a copy of said DNA target.

149. (Original) The method of claim 148 wherein said DNA targets are isolated from a biological source or said DNA targets are complete or partial copies of nucleic acids isolated from a biological source.

150. (Original) The method of claim 149 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from a biological source.

151. (Original) The method of claim 149 wherein a non-inherent UDT has been added during the copying of said target DNA.

152. (Original) The method of claim 148 wherein said addition reagents comprise:

- a) a ligase; or
- b) Terminal Deoxynucleotidyl Transferase.

153. (Original) The method of claim 148 wherein said modifying reagents comprise reagents for periodate oxidation of the 3' ends.

154. (Original) The method of claim 151 wherein said primer comprises a sequence complementary to a non-inherent UDT.

155. (Original) The method of claim 148 wherein said inherent UDTs comprise poly A segments or consensus segments.

156. (Original) The method of claim 154 wherein said inherent UDTs comprise 3' poly A segments or consensus segments.

157. (Original) The composition of claim 156 wherein said consensus segments comprise signal sites for poly A addition, splicing elements, and multicopy repeats.
158. (Original) The method of claim 148 wherein an RNA promoter sequence has been added during the copying of said target DNA.
159. (Original) The method of claim 149 wherein said DNA targets comprise an RNA promoter sequence.
160. (Original) The method of claim 158 or 159 further comprising the steps of:
- providing reagents for RNA transcription; and
  - carrying out said RNA transcription.
161. (Original) The method of claim 158 or 159 further comprising the steps of:
- providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - providing dNTPs or a mixture of dNTPs and NTPs; and
  - creating a DNA transcript or a DNA/RNA chimeric transcript.
162. (Original) A composition of matter comprising a chimeric primer or chimeric nucleic acid construct wherein said chimeric primer or chimeric nucleic acid construct comprises:
- at least one deoxyribonucleotide; and
  - at least one ribonucleotide at the 3' terminus.
163. (Original) The composition of claim 162 wherein said chimeric primer or chimeric nucleic acid construct further comprises a sequence for a production center.
164. (Original) The composition of claim 163 wherein said production center comprises an RNA promoter sequence.

165.(Original) The composition of claim 164 wherein said RNA promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.

166. (Original) The composition of claim 163 wherein said chimeric primer or chimeric nucleic acid construct comprises sequences complementary to an inherent UDT or a non-inherent UDT.

167. (Original) The composition of claim 166 wherein said inherent UDT comprises 3' poly A segments or consensus segments.

168. (Original) The composition of claim 167 wherein said consensus segments comprise signal sites for poly A addition, splicing elements or multicopy repeats.

169. (Original) The composition of claim 162 wherein said chimeric primer or chimeric nucleic acid construct is attached to a solid matrix.

170. (Original) The composition of claim 162 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

171. (Original) A composition of matter comprising:

a) a primer or nucleic acid construct wherein said primer or nucleic acid construct comprises:

(i) at least one deoxyribonucleotide; and

(ii) at least one ribonucleotide or at least one nucleotide analogue at the 3' terminus; and

b) a solid matrix attached to said primer or nucleic acid construct.

172. (Original) The composition of claim 171 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

173. (Original) The composition of claim 171 wherein said deoxyribonucleotides comprise a homopolymeric segment of at least twelve nucleotides.

174. (Original) The composition of claim 173 wherein the sequence of said homopolymeric segment comprises T, U or any combination thereof.

175. (Original) The composition of claim 173 wherein the sequence of said homopolymeric segment comprises oligo-C, oligo-G or oligo A.

176. (Original) The composition of claim 171 wherein said nucleotide analogue contains a substitution in the 2' position.

177. (Original) The composition of claim 176 wherein said nucleotide analogue comprises 2' O-methyl, 2' Fluoro or 2' amino nucleotide analogues.

178. (Original) A composition of matter comprising:

- a) a primer or nucleic acid construct wherein said primer or nucleic acid construct comprises a set of permutational primers or nucleic acid constructs with the formula 5'  $H_x-N_yN_z-3'$  wherein  $H_x$  is a homopolymeric nucleotide sequence of a single base "H";  $x$  is an integer between 10 and 30;  $N_y$  comprises a single base selected from a mixture of all bases other than the base "H" of said homopolymeric nucleotide sequence;  $N_z$  comprises a single base selected from a mixture of all four bases and  $N_z$  also comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position; and
- b) a solid matrix attached to said primer or nucleic acid construct.

179. (Original) The composition of claim 178 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

180. (Original) A composition of matter comprising a primer or nucleic acid construct wherein said primer or nucleic acid construct comprises:

- a) a homopolymeric segment wherein said homopolymeric segment comprises at least 12 nucleotides; and
- b) at least one nucleotide analogue with a substitution in the 2' position, wherein said nucleotide analogue is at the 3' end of said primer or nucleic acid construct.

181. (Original) The composition of claim 180 wherein the sequence of said homopolymeric segment comprises T, U or any combination thereof.

182. (Original) The composition of claim 180 wherein the sequence of said homopolymeric segment comprises oligo-C, oligo-G or oligo A.

183. (Original) The composition of claim 180 wherein said nucleotide analogue comprises a portion of said homopolymeric segment.

184. (Original) The composition of claim 180 wherein the base of at least one nucleotide analogue is different from the bases comprising said homopolymeric segment.

185. (Original) The composition of claim 180 wherein said nucleotide analogue comprises 2' O-methyl, 2' Fluoro or 2' amino nucleotide analogues.

186. (Original) The composition of claim 180 wherein said primer or nucleic acid construct further comprises a sequence for a production center.

187. (Original) The composition of claim 186 wherein said production center comprises an RNA promoter sequence.

188. (Original) The composition of claim 187 wherein said promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.



189. (Original) The composition of claim 180 wherein said primer or nucleic acid construct is attached to a solid matrix.
190. (Original) The composition of claim 189 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.
191. (Original) A set of permutational primers or nucleic acid constructs with the formula  $5' H_x-N_yN_z-3'$  wherein  $H_x$  is a homopolymeric nucleotide sequence comprising a single base "H";  $x$  is an integer between 10 and 30;  $N_y$  comprises a single base selected from a mixture of all bases other than the base "H" of said homopolymeric nucleotide sequence;  $N_z$  comprises a single base selected from a mixture of all four bases and  $N_z$  also comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position.
192. (Original) The method of claim 191 wherein  $N_y$  also comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position.
193. (Original) The sets of permutational primers or nucleic acid constructs of claim 191 or 192, wherein  $N_z$  is omitted and  $N_y$  comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position.
194. (Original) The sets of permutational primers or nucleic acid constructs of claim 191, 192, or 193 wherein  $H_x$  comprises T, U or a combination thereof and  $N_y$  comprises A, G or C.
195. (Original) The sets of permutational primers or nucleic acid constructs of claims 191, 192, or 193, wherein the homopolymeric nucleotide sequence  $H_x$  comprises oligo-C, oligo-G or oligo A.

196. (Original) The sets of of permutational primers or nucleic acid constructs of claims 191, 192 or 193, wherein said nucleotide analogue with a substitution in the 2' position comprises 2' O-methyl, 2' Fluoro or 2' amino nucleotide analogues.
197. (Original) The sets of of permutational primers or nucleic acid constructs of claims 191, 192 or 193, wherein said primers or nucleic acid constructs further comprise a sequence for a production center.
198. (Original) The sets of of permutational primers or nucleic acid constructs of claim 197 wherein said production center comprises an RNA promoter sequence
199. (Original) The sets of of permutational primers or nucleic acid constructs of claim 198 wherein said promoter sequences code for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase
200. (Original) The sets of of permutational primers or nucleic acid constructs of claims 191, 192 or 193, wherein said primer or nucleic acid constructs are attached to a solid matrix.
201. (Original) The sets of of permutational primers or nucleic acid constructs of claim 200, wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.
202. (Original) A set of primers or nucleic acid constructs with the formula 5' Pro-H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' wherein Pro comprises a nucleotide sequence for an RNA promoter; H<sub>x</sub> is a homopolymeric nucleotide sequence comprising a single base "H"; x is an integer between 10 and 30; N<sub>y</sub> comprises a single base selected from a mixture of all bases other than the base "H" of said homopolymeric nucleotide sequence; N<sub>z</sub> comprises a single base from a mixture of all four bases and N<sub>z</sub> also comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position.

203. (Original) The method of claim 202 wherein  $N_y$  also comprises a ribonucleotide or a nucleotide analogue with a substitution in the 2' position.
- 204 (Original) The set of permutational primers or nucleic acid constructs of claim 202 wherein  $N_z$  is omitted and  $N_y$  comprises a nucleotide or a nucleotide analogue with a substitution in the 2' position
205. (Original) The sets of permutational primers or nucleic acid constructs of claims 202, 203 or 204 wherein  $H_x$  comprises T, U or a combination thereof and  $N_y$  comprises A, G or C.
206. (Original) The sets of permutational primers or nucleic acid constructs of claims 202, 203 or 204 wherein said homopolymeric nucleotide sequence  $H_x$  comprises oligo-C, oligo-G or oligo-A.
207. (Original) The sets of permutational primers or nucleic acid constructs of claims 202, 203 or 204 wherein said nucleotide analogue with a substitution in the 2' position comprises 2' O-methyl, 2' Fluoro and 2' amino nucleotide analogues.
208. (Original) The sets of permutational primers or nucleic acid constructs of claim 202, 203 or 204 wherein said RNA promoter codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.
209. (Original) The sets of permutational primers or nucleic acid constructs of claims 202, 203 or 204 wherein said primer or nucleic acid constructs are attached to a solid matrix.
210. (Original) The sets of permutational primers or nucleic acid constructs of claim 209 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

211. (Original) A method for synthesizing at least one copy of a library of nucleic acid targets that comprises the steps of:

- a) providing:
  - (i) a library of nucleic acid targets;
  - (ii) primers or nucleic acid constructs comprising sequences complementary to homopolymeric sequences in said library of nucleic acid targets wherein said primers or nucleic acid constructs comprise one or more terminal nucleotides at their 3' ends, wherein said terminal nucleotides comprise nucleotide analogues with substitutions on the 2' position of the ribose ring; and
  - (iii) synthesizing reagents for synthesis of a nucleic acid copy;
- b) annealing said primers or nucleic acid constructs to said homopolymeric sequences in said library of nucleic acid targets; and
- c) extending the annealed primers or nucleic acid constructs by said synthesizing reagents using said nucleic acids as templates for the synthesis of at least one nucleic acid copy of all or a portion of said library of nucleic acid targets.

212. (Original) The method of claim 211 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acid targets are complete or partial copies of nucleic acids isolated from a biological source.

213. (Original) The method of claim 212 wherein said complete or partial copies of nucleic acids are identical or complementary copies of said nucleic acids isolated from a biological source.

214. (Original) The method of claim 212 wherein said homopolymeric sequences are present in said library of nucleic acid targets prior to or after said isolation of said library of nucleic acid targets from said biological source.

215. (Original) The method of claim 214 wherein said homopolymeric sequences comprise Poly A sequences.

216. (Original) The method of claim 212 wherein said homopolymeric sequences are added to said library of nucleic acid targets by an enzyme after isolation of said library of nucleic acid targets from said biological source.

217. (Original) The method of claim 213 wherein said homopolymeric sequences are added to said identical or complementary copies during or after preparation of said copies.

218. (Original) The method of claim 216 wherein said enzyme adding the homopolymeric sequences to the nucleic acid targets comprises Terminal Deoxynucleotidyl Transferase or a ligase.

219. (Original) The method of claim 211 wherein said nucleotide analogues comprise 2' O-methyl analogues, 2' Fluoro analogues or 2' amino analogues.

220. (Original) The method of claim 211 wherein said primers or nucleic acid constructs are chimeric and comprise nucleotides other than 2' substituted nucleotide analogues.

221. (Original) The method of claim 211 wherein said synthesizing reagents comprise E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript, any mutational variations of any of the preceding, or any combination of the preceding.

222. (Original) The method of claim 211 further comprising the step of removing or fragmenting said templates and synthesizing a complementary copy of said nucleic acid copy.

223. (Original) The method of claim 222 wherein said complementary copy is formed by a partial RNase H digestion of an RNA template, hairpin formation at the 3' terminus or by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said nucleic acid copy.

224. (Original) The method of claim 211 wherein a non-inherent UDT is added to said nucleic acid copy.

225. (Original) The method of claim 224 further comprising the step of synthesizing a complementary copy of said nucleic acid copy.

226. (Original) The method of claim 224 wherein the reagent for said addition of said non-inherent UDT comprises Terminal Deoxynucleotidyl Transferase or a ligase.

227. (Original) The method of claim 226 further comprising the step of adding a terminator nucleotide.

228. (Original) The method of claim 224 wherein said non-inherent UDT and a terminator nucleotide are added to said nucleic acid copy by providing Terminal Deoxynucleotidyl Transferase and a mixture of terminator and non-terminator nucleotides.

229. (Original) The method of claim 227 or 228 further comprising the step of synthesizing a complementary copy of said nucleic acid copy.

230. (Original) The method of claim 227 or 228 wherein said terminator nucleotides comprise dideoxyribonucleotides, acyclic nucleotides, arabinosides or 3' amino nucleotides.

231. (Original) The method of claim 211 wherein said primers or said nucleic acid constructs comprise an RNA promoter sequence.
232. (Original) The method of claim 219 wherein said primers or said nucleic acid constructs comprise a production center.
233. (Original) The method of claim 223 wherein said reverse primers or reverse nucleic acid constructs comprise a production center.
234. (Original) The method of claim 232 or 233 wherein said production center comprises an RNA promoter sequence.
235. (Original) The method of claim 234 wherein said RNA promoter sequence comprises T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter sequences.
236. (Original) The method of claim 234 further comprising the steps of:
- a) providing appropriate reagents; and
  - b) carrying out a transcription reaction using said RNA promoter sequence.
237. (Original) The method of claim 234 further comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs or NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
238. (Original) The method of claim 237 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.

239. (Original) The method of claim 237 wherein said DNA transcript or DNA, RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.

240. (Original) The method of claim 237 further comprising the step of synthesizing cDNA in the presence at least one labeled nucleotide, thereby generating labeled cDNA products.

241. (Original) The method of claim 238 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

242. (Original) The method of claim 239 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

243. (Original) The method of claim 240 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

244. (Original) The method of claim 211 wherein said library of nucleic acid targets comprises DNA or RNA.



245. (Original) The method of claim 211 wherein said primer or nucleic acid construct is attached to a solid matrix.

246. (Original) The method of claim 245 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

247. (Original) The method of claim 211 wherein the sequence of said homopolymeric segment is comprised of T, U or any combination thereof.

248. (Original) The method of claim 211 wherein the sequence of said homopolymeric segment comprises oligo-C, oligo-G or oligo-A.

249. (Original) The method of claim 211 wherein one or more of said nucleotide analogues comprise a portion of said homopolymeric sequence.

250. (Original) The method of claim 211 wherein one or more of the bases of said nucleotide analogues are different from the bases comprising said homopolymeric segment.

251. (Original) A method for synthesizing one or more copies of a library of nucleic acid targets that comprises the steps of:

- a) providing:
  - (i) a library of nucleic acid targets;
  - (ii) primers or nucleic acid constructs comprising sequences complementary to homopolymeric sequences in said library of nucleic acid targets wherein said primers or nucleic acid constructs comprise one or more terminal nucleotides at their 3' ends, wherein said terminal nucleotides comprise nucleotide analogues with substitutions on the 2' position of the ribose ring;
  - (iii) synthesizing reagents for the synthesis of a nucleic acid copy; and

- (iv) addition reagents for the addition of a non-inherent UDT; and
- b) annealing said primers or nucleic acid constructs to said homopolymeric sequences in said library of nucleic acid targets;
- c) extending the annealed primers or nucleic acid constructs by said synthesizing reagents for the synthesis of at least one nucleic acid copy of said library of nucleic acid targets; and
- d) adding a non-inherent UDT to said extended primers or said extended nucleic acid constructs.

252. (Original) The method of claim 251 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acids are complete or partial copies of nucleic acids isolated from a biological source.

253. (Original) The method of claim 252 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from said biological source.

254. (Original) The method of claim 252, wherein said homopolymeric sequences are present in said library of nucleic acid targets prior to or after said isolation of said library of nucleic acid targets from said biological source.

255. (Original) The method of claim 252 wherein said homopolymeric sequences are added to said library of nucleic acid targets by an enzyme after isolation of said library of nucleic acid targets from said biological source.

256. (Original) The method of claim 254, wherein said homopolymeric sequences comprise poly A sequences.

257 (Original) The method of claim 253 wherein said homopolymeric sequences are added to said identical or complementary copies during or after preparation of said copies.

258. (Original) The method of claim 255, wherein said enzyme adding the homopolymeric sequences to the nucleic acid targets comprises poly A polymerase, Terminal Deoxynucleotidyl Transferase or a ligase.
259. (Original) The method of claim 251, wherein said nucleotide analogues comprise 2' O-methyl analogues, 2' Fluoro analogues or 2' amino analogues.
260. (Original) The method of claim 251, wherein said primers or nucleic acid constructs are chimeric and comprise nucleotides other than 2' substituted nucleotide analogues.
261. (Original) The method of claim 251 wherein said synthesizing reagents comprise E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\square$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript, any mutational variations of any of the preceding, or any combination of the preceding.
262. (Original) The method of claim 251 further comprising the steps of:
- providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
  - separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
  - synthesizing said complementary copy.
263. (Original) The method of claim 262, wherein said complementary copy is formed by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said non-inherent UDT.

264. (Original) The method of claim 251, wherein said addition reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.
265. (Original) The method of claim 264, further comprising the step of adding a terminator nucleotide.
266. (Original) The method of claim 251, wherein said non-inherent UDT and a terminator nucleotide are added to said nucleic acid copy by providing Terminal Deoxynucleotidyl Transferase and a mixture of terminator and non-terminator nucleotides.
267. (Original) The method of claim 266 further comprising the steps of:
- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
  - b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
  - c) synthesizing said complementary copy.
268. (Original) The method of claim 265 or 266, wherein said terminator nucleotides comprise dideoxyribonucleotides, acyclic nucleotides, arabinosides or 3' amino nucleotides.
269. (Original) The method of claim 251, wherein said primers or said nucleic acid constructs comprise a production center.
270. (Original) The method of claim 263, wherein said reverse primers or reverse nucleic acid constructs comprise a production center.
271. (Original) The method of claim 269 or 270 wherein said production center comprises an RNA promoter sequence.

272. (Original) The method of claim 271 wherein said RNA promoter sequence comprises T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter sequences.
273. (Original) The method of claim 271, further comprising the steps of:
- a) providing appropriate reagents; and
  - b) carrying out a transcription reaction using said RNA promoter sequence.
274. (Original) The method of claim 271 further comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising Mn<sup>++</sup>, mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or dNTPs and NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
275. (Original) The method of claim 273 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
276. (Original) The method of claim 274 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
277. (Original) The method of claim 273 further comprising the step of synthesizing a nucleic acid copy in the presence of at least one labeled nucleotides, thereby generating labeled nucleic acid copy products.
278. (Original) The method of claim 275 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

279. (Original) The method of claim 276 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

280. (Original) The method of claim 277 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

281. (Original) The method of claim 251 wherein said library of nucleic acid targets comprises DNA or RNA.

282. (Original) The method of claim 251 wherein said primer or nucleic acid construct is attached to a solid matrix.

283. (Original) The method of claim 289 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

284. (Original) The method of claim 251 wherein said sequence of said homopolymeric segment is comprised of T, U or any combination thereof.

285. (Original) The method of claim 251 wherein said sequence of said homopolymeric segment comprises oligo-C, oligo-G or oligo-A.

286. (Original) The method of claim 251 wherein at least one of said nucleotide analogues comprise a portion of said homopolymeric sequence.
287. (Original) The method of claim 251 wherein at least one of said bases of said nucleotide analogues are different from the bases comprising said homopolymeric segment.
288. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:
- a) providing:
    - (i) at least one nucleic acid target;
    - (ii) at least one primer or nucleic acid construct complementary to a sequence in said nucleic acid target, wherein said primer or nucleic acid construct is a chimeric primer or chimeric nucleic acid construct comprising at least one deoxyribonucleotide and at least one ribonucleotide wherein at least one of said ribonucleotides is at the 3' terminus of said chimeric primer or said chimeric nucleic acid construct; and
    - (iii) synthesizing reagents for the synthesis of a nucleic acid copy;
  - b) annealing said chimeric primer or chimeric nucleic acid construct to said nucleic acid target; and
  - c) extending said chimeric primer or chimeric nucleic acid construct by said synthesizing reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target.
289. (Original) The method of claim 288 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acids are complete or partial copies of nucleic acids isolated from a biological source.
290. (Original) The method of claim 289 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from said biological source.

291. (Original) The method of claim 288 wherein said synthesizing reagents comprise E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript, any mutational variations of any of the preceding, or any combination of the preceding.

292. (Original) The method of claim 288 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said cDNA copy;
- b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
- c) synthesizing said complementary copy.

293. (Original) The method of claim 292, wherein said complementary copy is formed by a partial RNase H digestion of an RNA template, hairpin formation at the 3' terminus of said cDNA copy or by providing at least one reverse primer or reverse nucleic acid construct comprising sequences complementary to sequences in said cDNA copy.

294. (Original) The method of claim 293 further comprising:

- a) providing:
  - (i) reagents for the addition of a non-inherent UDT;
- b) adding said non-inherent UDT to said cDNA copy wherein said reverse primers or reverse nucleic acid constructs are complementary to sequences in said non-inherent UDT.

295. (Original) The method of claim 288 wherein said primer or said nucleic acid construct comprises a production center.



296. (Original) The method of claim 293 wherein said reverse primers or reverse nucleic acid constructs comprise a production center.
297. (Original) The method of claim 295 or 296 wherein said production center comprises an RNA promoter sequence.
298. (Original) The method of claim 297 wherein said RNA promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.
299. (Original) The method of claim 295 wherein said primer or said nucleic acid construct comprises sequences complementary to an inherent UDT or a non-inherent UDT.
300. (Original) The method of claim 299 wherein said inherent UDT comprises 3' poly A segments or consensus segments.
301. (Original) The method of claim 300 wherein said consensus segments comprise signal sites for poly A addition, splicing elements, and multicopy repeats.
302. (Original) The method of claim 297 further comprising the steps of:
- providing appropriate reagents; and
  - carrying out a transcription reaction using said RNA promoter sequence.
303. (Original) The method of claim 297 further comprising the steps of:
- providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - providing dNTPs or a mixture of dNTPs or NTPs; and
  - creating a DNA transcript or a DNA/RNA chimeric transcript.

304. (Original) The method of claim 302 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotides thereby generating transcription products.

305. (Original) The method of claim 303 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.

306. (Original) The method of claim 302 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.

307. (Original) The method of claim 304 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

308. (Original) The method of claim 305 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

309. (Original) The method of claim 306 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a

hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

310. (Original) The method of claim 288 wherein said primer or nucleic acid construct is attached to a solid matrix.

311. (Original) The method of claim 310 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

312. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) at least one chimeric primer or chimeric nucleic acid construct comprising sequences complementary to a segment of said nucleic acid target sequence, wherein said chimeric primer or chimeric nucleic acid construct comprises at least one deoxyribonucleotide and at least one nucleotide other than a deoxyribonucleotide at the 3' terminus of said chimeric primer or chimeric nucleic acid construct;
  - (iii) template dependent reagents for the addition of nucleotides; and
  - (iv) template independent reagents for the addition of nucleotides;
- b) annealing said nucleic acid target with said chimeric primer or chimeric nucleic acid construct;
- c) extending said annealed chimeric primer or annealed chimeric nucleic acid construct by said template dependent reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target; and
- d) adding on further nucleotides to said extended chimeric primer or chimeric nucleic acid construct with said template independent reagents under conditions that said further nucleotides are added to the 3' end of said extended chimeric primer or chimeric nucleic acid construct more efficiently than to the 3' end of

any chimeric primers or chimeric nucleic acid constructs that remain unextended.

313. (Original) The method of claim 312, wherein at least one nucleotide other than a deoxyribonucleotide comprises ribonucleotides or 2' substituted nucleotide analogues.

314. (Original) The method of claim 313 wherein said 2' substituted nucleotide analogues comprise 2' Fluoro, 2' O-methoxy and 2' amino nucleotide analogues.

315. (Original) The method of claim 312 wherein said nucleic acid targets are isolated from a biological source or said library of nucleic acids are complete or partial copies of nucleic acids isolated from a biological source.

316. (Original) The method of claim 315 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from said biological source.

317. (Original) The method of claim 312 wherein said chimeric primer or chimeric nucleic acid construct further comprises at least one additional nucleotide other than a deoxyribonucleotide.

318. (Original) The method of claim 317 wherein said additional nucleotide comprises a ribonucleotide or 2' substituted nucleotide analogue.

319. (Original) The method according to claim 312 wherein said template dependent reagents comprise E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript, any mutational variations of any of the preceding, or any combinations of the preceding.

320. (Original) The method according to claim 312 wherein said template independent reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.
321. (Original) The method of claim 312 further comprising the steps of synthesizing a complementary copy of said nucleic acid copy.
322. (Original) The method of claim 321 wherein said synthesis of a complementary copy comprises the steps of:
- a) providing:
    - (i) additional template dependent reagents; and
    - (ii) a reverse primer or reverse nucleic acid construct;
  - b) forming a reverse primer binding site by said addition of further nucleotides to said extended chimeric primer or chimeric nucleic acid construct;
  - c) annealing said reverse primer or reverse nucleic acid construct binding site with said reverse primer; and
  - d) extending said annealed reverse primer or reverse nucleic acid construct by said template dependent reagents to synthesize a complementary copy of said nucleic acid copy.
323. (Original) A method according to claim 312 or 322 wherein said nucleic acid target comprises an inherent or non-inherent UDT.
324. (Original) A method according to claim 323 wherein said chimeric primer or chimeric nucleic acid construct is complementary to said inherent or non-inherent UDT.
325. (Original) The method of claim 312 wherein said primer or said nucleic acid construct comprises a production center.

326. (Original) The method of claim 322 wherein said reverse primers or reverse nucleic acid constructs comprise a production center.
327. (Original) The method of claim 325 or 326 wherein said production center comprises an RNA promoter sequence.
328. (Original) The method of claim 327 wherein said RNA promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.
329. (Original) The method of claim 327 further comprising the steps of:
- a) providing appropriate reagents; and
  - b) carrying out a transcription reaction using said RNA promoter sequence.
330. (Original) The method of claim 327 further comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs or NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
331. (Original) The method of claim 329 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotides thereby generating transcription products.
332. (Original) The method of claim 330 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
333. (Original) The method of claim 329 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.

334. (Original) The method of claim 331 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.
335. (Original) The method of claim 332 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.
336. (Original) The method of claim 333 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.
337. (Original) The method of claim 331, 332 or 333 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.
338. (Original) A method according to claim 312 or 322 wherein said chimeric primer or chimeric nucleic acid construct is attached to a solid matrix.
339. (Original) A method according to claim 338 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates and glass slides.

340. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:
- a) providing
    - (i) at least one nucleic acid target;
    - (ii) at least one chimeric primer or chimeric nucleic acid construct comprising sequences complementary to a homopolymeric sequence in said nucleic acid target, wherein said chimeric primer or chimeric nucleic acid construct comprises at least one deoxyribonucleotide and at least one nucleotide other than a deoxyribonucleotide wherein at least one of said other nucleotides is at the 3' terminus of said chimeric primer or chimeric nucleic acid construct;
    - (v) template dependent reagents for the synthesis of a nucleic acid copy; and
    - (vi) template independent reagents for the addition of nucleotides;
  - b) annealing said chimeric primer or chimeric nucleic acid construct to said homopolymeric sequence in said nucleic acid target;
  - c) extending said annealed chimeric primer or annealed chimeric nucleic acid construct by said template dependent reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target;
  - d) adding on further nucleotides to said extended chimeric primer or chimeric nucleic acid construct with said template independent reagents under conditions that said further nucleotides are added to the 3' end of said extended chimeric primer or chimeric nucleic acid construct more efficiently than to the 3' end of said unextended chimeric primer or unextended chimeric nucleic acid construct.
341. (Original) The method of claim 340, wherein at least one nucleotide other than a deoxyribonucleotide comprises ribonucleotides or 2' substituted nucleotide analogues.
342. (Original) The method of claim 341 wherein said 2' substituted nucleotide analogues comprise 2' Fluoro, 2' O-methoxy and 2' amino nucleotide.



343. (Original) A method according to claim 340 wherein said homopolymeric sequence comprises a Poly A sequence, oligo-A, oligo-G, oligo-C or oligo-T.
344. (Original) The method of claim 340 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acid targets are complete or partial copies of nucleic acids isolated from a biological source.
345. (Original) The method of claim 344 wherein said complete or partial copies of nucleic acids are identical or complementary copies of said nucleic acids isolated from a biological source.
346. (Original) The method of claim 344 wherein said homopolymeric sequences are present in said library of nucleic acid targets prior to or after said isolation of said library of nucleic acid targets from said biological source.
347. (Original) The method of claim 344 wherein said homopolymeric sequences are added to said library of nucleic acid targets by an enzyme after isolation of said library of nucleic acid targets from said biological source.
348. (Original) The method of claim 345 wherein said homopolymeric sequences are added to said identical or complementary copies during or after preparation of said copies.
349. (Original) The method of claim 347 wherein said enzyme adding said homopolymeric sequence to said nucleic acid target comprises Terminal Deoxynucleotidyl Transferase or a ligase.
350. (Original) The method according to claim 340 wherein said template independent reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.

351. (Original) The method of claim 340 wherein said chimeric primers or nucleic acid constructs comprise nucleotides other than 2' substituted analogues.
352. (Original) The method of claim 340 wherein said chimeric primer or chimeric nucleic acid construct further comprises at least one additional nucleotide other than a deoxyribonucleotide.
353. (Original) The method of claim 352 wherein said additional nucleotide comprises a ribonucleotide or 2' substituted nucleotide analogue.
354. (Original) The method of claim 340 wherein said template dependent reagents comprise at least one of the following enzymes: E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript or any mutational variations of any of the preceding.
355. (Original) The method of claim 340 further comprising the step of synthesizing a complementary copy of said nucleic acid copy.
356. (Original) The method of claim 355 wherein said synthesis of a complementary copy comprises the steps of:
- a) providing:
    - (i) additional template dependent reagents;
    - (ii) a reverse primer or nucleic acid construct;
  - b) forming a reverse primer binding site by said addition of further nucleotides to said extended chimeric primer or chimeric nucleic acid construct;
  - c) annealing said reverse primer or nucleic acid construct binding site with said reverse primer; and

- d) extending said annealed reverse primer or nucleoc acid construct by said template dependent reagents to create a complementary copy.
- 357. (Original) A method according to claim 340 or 356 wherein said nucleic acid target comprises an inherent or non inherent UDT.
- 358. (Original) A method according to claim 357 wherein said chimeric primer or chimeric nucleic acid construct comprises sequences complementary to said inherent or non-inherent UDT.
- 359. (Original) The method of claim 340 wherein said primer or said nucleic acid construct comprises a production center.
- 360. (Original) The method of claim 356 wherein said reverse primers or reverse nucleic acid constructs comprise a production center.
- 361. (Original) The method of claim 359 or 360 wherein said production center comprises an RNA promoter sequence.
- 362. (Original) The method of claim 361 wherein said RNA promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.
- 363. (Original) The method of claim 361 further comprising the steps of:
  - a) providing appropriate reagents; and
  - b) carrying out a transcription reaction using said RNA promoter sequence.
- 364. (Original) The method of claim 361 further comprising the steps of:
  - a) providing reagents for RNA transcription, said reagents comprising Mn<sup>++</sup>, mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs or NTPs; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.

365. (Original) The method of claim 363 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotides thereby generating transcription products.

366. (Original) The method of claim 364 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.

367. (Original) The method of claim 363 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.

368. (Original) The method of claim 365 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

369. (Original) The method of claim 366 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

370. (Original) The method of claim 367 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a

haptens, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

371. (Original) The method of claim 365, 366 or 367 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.
372. (Original) A method according to claim 340 or 356 wherein said chimeric primer or chimeric nucleic acid construct is attached to a solid matrix.
373. (Original) A method according to claim 372 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates and glass slides.
374. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:
  - a) providing
    - (i) at least one nucleic acid target;
    - (ii) at least one chimeric primer or chimeric nucleic acid construct comprising sequences complementary to a segment of said nucleic acid target sequence, wherein said chimeric primer or chimeric nucleic acid construct comprises at least one deoxyribonucleotide and at least one nucleotide other than a deoxyribonucleotide wherein at least one of said other nucleotides is at the 3' terminus of said chimeric primer or chimeric nucleic acid construct;
    - (iii) template dependent reagents for the synthesis of a nucleic acid copy; and
    - (iv) template independent reagents for nucleic acid synthesis;
  - b) annealing said nucleic acid target with said chimeric primer or chimeric nucleic acid construct;
  - c) extending said annealed chimeric primer or annealed chimeric nucleic acid construct by said template dependent reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target; and

- d) adding on further nucleotides to said extended chimeric primer or chimeric nucleic acid construct to form a non-inherent UDT with said template independent reagents under conditions that said further nucleotides are added to the 3' ends of said extended chimeric primer or chimeric nucleic acid construct more efficiently than to the 3' ends of said unextended chimeric primer or unextended chimeric nucleic acid construct.
375. (Original) The method of claim 374, wherein at least one nucleotide other than a deoxyribonucleotide comprises ribonucleotides or 2' substituted nucleotide analogues.
376. (Original) The method of claim 375 wherein said 2' substituted nucleotide analogues comprise 2' fluoro, 2' o-methoxy and 2' amino nucleotide analogues.
377. (Original) The method of claim 374 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acid targets are complete or partial copies of nucleic acids isolated from a biological source.
378. (Original) The method of claim 377 wherein said complete or partial copies of nucleic acids are identical or complementary copies of said nucleic acids isolated from a biological source.
379. (Original) The method of claim 377 wherein said homopolymeric sequences are present in said library of nucleic acid targets prior to or after said isolation of said library of nucleic acid targets from said biological source.
380. (Original) The method of claim 377 wherein said homopolymeric sequences are added to said library of nucleic acid targets by an enzyme after isolation of said library of nucleic acid targets from said biological source.

381. (Original) The method of claim 378 wherein said homopolymeric sequences are added to said identical or complementary copies during or after preparation of said copies.
382. (Original) The method of claim 374 wherein said chimeric primer or chimeric nucleic acid construct further comprises at least one additional nucleotide other than a deoxyribonucleotide.
383. (Original) The method of claim 382 wherein said additional nucleotide comprises a ribonucleotide or a 2' substituted nucleotide analogue.
384. (Original) The method according to claim 374 wherein said template dependent reagents comprise at least one of the following enzymes: E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript or any mutational variations of any of the preceding.
385. (Original) The method according to claim 374 wherein said template independent reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.
386. (Original) The method of claim 374 further comprising the steps of:
- a) providing:
    - (i) additional template dependent reagents;
    - (ii) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said non-inherent UDT;
  - b) annealing said reverse primer or reverse nucleic acid construct to said UDT; and

- c) extending said annealed reverse primer or reverse nucleic acid construct by said additional template dependent reagents to synthesize a complementary copy of said nucleic acid copy.
387. (Original) The method of claim 374 or 386 wherein said nucleic acid target comprises an inherent or non-inherent UDT.
388. (Original) The method of claim 387 wherein said chimeric primer or chimeric nucleic acid construct is complementary to said inherent or non-inherent UDT in said nucleic acid target.
389. (Original) The method of claim 374 wherein said chimeric primer or chimeric nucleic acid construct comprises an RNA promoter.
390. (Original) The method according to claim 386 wherein said reverse primer comprises an RNA promoter.
391. (Original) The method of claim 389 or 390 wherein said promoter sequence comprises T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter sequences.
392. (Original) A method according to claim 389 or 390 further comprising the steps of:
- a) providing appropriate reagents;
  - b) carrying out a transcription reaction using said promoter sequence.
393. (Original) The method of claim 389 or 390 comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising Mg<sup>++</sup>, mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs and NTPS; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.



394. (Original) The method of claim 392 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
395. (Original) The method of claim 393 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled DNA or labeled DNA/RNA chimeric transcription products.
396. (Original) The method of claim 392 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.
397. (Original) The method of claim 394 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.
398. (Original) The method of claim 395 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.
399. (Original) The method of claim 396 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an

intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

- 400. (Original) The method of claim 394, 395 or 396 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.
- 401. (Original) The method of claim 374 or 386 further comprising the step of rendering said extended chimeric primer and nucleic acid target into a single-stranded extended chimeric primer.
- 402. (Original) The method of claim 401 wherein said rendering step is carried out by enzymatic digestion.
- 403. (Original) The method of claim 402 wherein said enzymatic digestion comprises RNase digestion.
- 404. (Original) The method of claim 401 wherein said rendering is carried out by heat denaturation.
- 405. (Original) A method of claim 374 or 386 wherein said chimeric primer or chimeric nucleic acid construct is attached to a solid matrix.
- 406. (Original) A method according to claim 405 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates and glass slides.
- 407. (Original) A method according to claim 374 or 386 wherein said nucleic acid target comprises DNA or RNA.
- 408. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (v) at least one chimeric primer or chimeric nucleic acid construct complementary to a segment of said nucleic acid target sequence, wherein said chimeric primer or chimeric nucleic acid construct comprises at least one deoxyribonucleotide and at least one nucleotide other than a deoxyribonucleotide wherein at least one of said other nucleotides is at the 3' terminus of said chimeric primer or chimeric nucleic acid construct;
  - (vi) template dependent reagents for the synthesis of a nucleic acid copy; and
  - (vii) template independent reagents for nucleic acid synthesis;
- b) annealing said nucleic acid target with said chimeric primer or chimeric nucleic acid construct;
- c) extending said annealed chimeric primer or annealed chimeric nucleic acid construct by said template dependent reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target;
- d) rendering said extended chimeric primer and nucleic acid target into a single-stranded extended chimeric primer; and
- e) adding on further nucleotides to said extended chimeric primer or chimeric nucleic acid construct to form a non-inherent UDT with said template independent reagents under conditions that said further nucleotides are added to the 3' ends of said extended chimeric primer or chimeric nucleic acid construct more efficiently than to the 3' ends of said unextended chimeric primer or unextended chimeric nucleic acid construct.

409. (Original) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) at least one chimeric primer or chimeric nucleic acid construct complementary to a segment of said nucleic acid target sequence,

- wherein said chimeric primer or chimeric nucleic acid construct comprises at least one deoxyribonucleotide and at least one nucleotide other than a deoxyribonucleotide wherein at least one of said other nucleotides is at the 3' terminus of said chimeric primer or chimeric nucleic acid construct;
- (iii) template dependent reagents for the synthesis of a nucleic acid copy; and
  - (iv) template independent reagents for nucleic acid synthesis;
- b) annealing said nucleic acid target with said chimeric primer or chimeric nucleic acid construct;
  - c) extending said annealed chimeric primer or annealed chimeric nucleic acid construct by said template dependent reagents using said nucleic acid target as a template to synthesize a copy of said nucleic acid target;
  - d) adding on further nucleotides to said extended chimeric primer or chimeric nucleic acid construct to form a non-inherent UDT with said template independent reagents under conditions that said further nucleotides are added to the 3' ends of said extended chimeric primer or chimeric nucleic acid construct more efficiently than to the 3' ends of said unextended chimeric primer or unextended chimeric nucleic acid construct; and
  - e) rendering said extended chimeric primer and nucleic acid target into a single-stranded extended chimeric primer.

410. (Original) A method for synthesizing at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) a set of permutational primers or nucleic acid constructs with the formula 5' H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' wherein H<sub>x</sub> is a homopolymeric nucleotide sequence comprising a single base "H"; x is an integer between 10 and 30; N<sub>y</sub> comprises a single nucleotide selected from a mixture of all bases other than the base "H" of said homopolymeric nucleotide sequence; and N<sub>z</sub> comprises a single base from a mixture of all four bases, a ribonucleotide, or a nucleotide analogue with a substitution in the 2' position; and

- (iii) synthesizing reagents for the synthesis of a nucleic acid copy;
- b) contacting said nucleic acid target with said set of permutational primers or nucleic acid constructs; and
- c) extending said set of permutational primers or nucleic acid constructs by said synthesizing reagents using said nucleic acid targets as a template to synthesize a cDNA copy of said nucleic acid target.

411. (Original) The method of claim 410 wherein said set of permutational primers or nucleic acid constructs with the formula 5' H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' further comprises a heteropolymeric sequence at the 5' end.

412. (Original) The method of claim 410 wherein N<sub>z</sub> is omitted and N<sub>y</sub> comprises a nucleotide, or a nucleotide analogue with a substitution in the 2' position.

413. (Original) The method of claim 410 or 412 wherein H<sub>x</sub> comprises nucleotide T, U or a combination thereof and N<sub>y</sub> comprises nucleotide A, G or C.

414. (Original) The method of claim 410 or 412 wherein said homopolymeric nucleotide sequence H<sub>x</sub> comprises oligo-C, oligo-G or oligo A.

415. (Original) The method of claim 410 or 412 wherein said nucleotide analogue with a substitution in the 2' position comprises 2' o-methyl, 2' fluoro or 2' amino nucleotide analogues.

416. (Original) The method of claim 410 or 412 wherein said primers or nucleic acid constructs further comprise a sequence for a production center.

417. (Original) The method of claim 416 wherein said production center comprises an RNA promoter sequence.

418. (Original) The method of claim 417 wherein said promoter sequence codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.

419. (Original) The method of claim 410 wherein said primers or nucleic acid constructs are attached to a solid matrix.

420 (Original) The method of claim 419 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.

421. (Original) A method for synthesizing at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) a set of permutational primers or nucleic acid constructs with the formula 5' H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' wherein H<sub>x</sub> is a homopolymeric sequence comprising a single base or nucleotide analogue "H"; x is an integer between 10 and 30; N<sub>y</sub> comprises a single base or nucleotide analogue selected from a mixture of bases and nucleotide analogues other than the base or nucleotide analogue "H" of said homopolymeric nucleotide sequence; and N<sub>z</sub> comprises a single base from a mixture of all bases, a ribonucleotide, or a nucleotide analogue with a substitution in the 2' position; and
  - (iii) synthesizing reagents for the synthesis of a nucleic acid copy;
- b) contacting said nucleic acid target with said set of permutational primers or nucleic acid constructs; and
- c) extending said set of permutational primers or nucleic acid constructs by said synthesizing reagents using said nucleic acid targets as a template to synthesize a cDNA copy of said nucleic acid target.

422. (Original) The method of claim 421 wherein said set of permutational primers or nucleic acid constructs with the formula 5' H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' further comprises a heteropolymeric sequence at the 5' end.

423. (Original) The method of claim 421 wherein H<sub>x</sub> comprises nucleotide or nucleotide analogue T, U or a combination thereof and N<sub>y</sub> comprises nucleotide or nucleotide analogue A, G or C.
424. (Original) The method of claim 421 wherein the homopolymeric sequence H<sub>x</sub> comprises oligo-C, oligo-G or oligo A.
425. (Original) The method of claim 421 wherein said nucleotide analogue with a substitution in the 2' position comprises 2' o-methyl, 2' fluoro or 2' amino nucleotide analogues.
426. (Original) The method of claim 421 wherein said primer or nucleic acid constructs further comprise a sequence for a production center
427. (Original) The method of claim 426 wherein said production center comprises an RNA promoter sequence.
428. (Original) The method of claim 427 wherein said promoter sequences code for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.
429. (Original) The method of claim 421 wherein said primers or nucleic acid constructs are attached to a solid matrix.
430. (Original) The method of claim 429 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates or glass slides.
431. (Original) A method for synthesizing at least one nucleic acid target comprising the steps of:
- a) providing
    - (i) at least one nucleic acid target;

- (ii) a set of primers or nucleic acid constructs with the formula 5' Pro-H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' wherein Pro comprises a nucleotide sequence for an RNA promoter; H<sub>x</sub> is a homopolymeric nucleotide sequence comprising a single base "H"; x is an integer between 10 and 30; N<sub>y</sub> comprises a single base selected from a mixture of all bases other than the base "H" of said homopolymeric nucleotide sequence; and N<sub>z</sub> comprises a single base from a mixture of all four bases, a ribonucleotide, or a nucleotide analogue with a substitution in the 2' position; and
- (iii) synthesizing reagents for the synthesis of a nucleic acid copy;
- b) contacting said nucleic acid target with said set of permutational primers or nucleic acid constructs; and
- c) extending said set of permutational primers or nucleic acid constructs by said synthesizing reagents using said nucleic acid targets as a template to synthesize a cDNA copy of said nucleic acid target.

432. (Original) The method of claim 431 wherein said set of permutational primers or nucleic acid constructs with the formula 5' Pro-H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' further comprises a heteropolymeric sequence at the 5' end.

433. (Original) The method of claim 432 wherein N<sub>z</sub> is omitted and N<sub>y</sub> comprises a nucleotide or a nucleotide analogue with a substitution in the 2' position.

434. (Original) The method of claim 431 or 433 wherein H<sub>x</sub> comprises nucleotide T, U or a combination thereof and N<sub>y</sub> comprises a nucleotide A, G and C.

435. (Original) The method of claim 431 or 433 wherein the homopolymeric nucleotide sequence H<sub>x</sub> comprises oligo-C, oligo-G or oligo A.

436. (Original) The method of claim 431 or 433 wherein said nucleotide analogue with a substitution in the 2' position comprises 2' o-methyl, 2' fluoro and 2' amino nucleotide analogues.



437. (Original) The method of claim 431 or 433 wherein said RNA promoter codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.

438. (Original) The method of claim 431 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
- b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
- c) synthesizing said complementary copy.

439. (Original) The method of claim 438, wherein said complementary copy is formed by a partial RNase digestion of an RNA template, hairpin formation at the 3' terminus of said nucleic acid copy or by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said nucleic acid copy.

440. (Original) The method of claim 438 further comprising:

- a) providing:
  - (i) reagents for the addition of a non-inherent UDT to said first nucleic acid copy;
  - (ii) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT;
- b) adding said non-inherent UDT to said nucleic acid copy;
- c) annealing said reverse primer or reverse nucleic acid construct to said non-inherent UDT; and
- d) extending said annealed reverse primer or nucleic acid construct.

441. (Original) A method of claim 431 or 433 wherein said primer or nucleic acid constructs are attached to a solid matrix.

442. (Original) A method according to claim 441 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates and glass slides.

443. (Original) The method for synthesizing at least one nucleic acid target comprising the steps of:
- a) providing
    - (i) at least one nucleic acid target;
    - (ii) a set of primers or nucleic acid constructs with the formula 5' Pro-H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' wherein Pro comprises a nucleotide sequence for an RNA promoter; H<sub>x</sub> is a homopolymeric sequence of a single nucleotide or nucleotide analogue "H"; x is an integer between 10 and 30; N<sub>y</sub> comprises a single nucleotide selected from a mixture of nucleotides and nucleotide analogues other than the nucleotide or nucleotide analogue "H" of said homopolymeric nucleotide sequence; and N<sub>z</sub> comprises a single nucleotide from a mixture of all nucleotides, a ribonucleotide, or a nucleotide analogue with a substitution in the 2' position;
    - (iii) synthesizing reagents for the synthesis of a nucleic acid copy
  - b) contacting said nucleic acid target with said set of permutational primers or nucleic acid constructs; and
  - c) extending said set of permutational primers or nucleic acid constructs by said synthesizing reagents using said nucleic acid targets as a template to synthesize a cDNA copy of said nucleic acid target.
444. (Original) The method of claim 443 wherein said set of permutational primers or nucleic acid constructs with the formula 5' Pro-H<sub>x</sub>-N<sub>y</sub>N<sub>z</sub>-3' further comprises a heteropolymeric sequence at the 5' end.
445. (Original) The method of claim 443 wherein H<sub>x</sub> comprises nucleotide T, U or a combination thereof and N<sub>y</sub> comprises a nucleotide A, G and C.
446. (Original) The method of claim 443 wherein the homopolymeric nucleotide sequence H<sub>x</sub> comprises oligo-C, oligo-G or oligo A.

447. (Original) The method of claim 443 wherein said nucleotide analogue with a substitution in the 2' position comprises 2' o-methyl, 2' fluoro and 2' amino nucleotide analogues.

448. (Original) The method of claim 443 or 445 wherein said RNA promoter codes for transcription by T3 RNA polymerase, T7 RNA polymerase or SP6 RNA polymerase.

449. (Original) The method of claim 443 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
- b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
- c) synthesizing said complementary copy.

450. (Original) The method of claim 449, wherein said complementary copy is formed by a partial RNase digestion of an RNA template, hairpin formation at the 3' terminus of said nucleic acid copy or by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said nucleic acid copy.

451. (Original) The method of claim 449 further comprising:

- a) providing:
  - (i) reagents for the addition of a non-inherent UDT to said first nucleic acid copy;
  - (ii) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT;
- b) adding said non-inherent UDT to said nucleic acid copy;
- c) annealing said reverse primer or reverse nucleic acid construct to said non-inherent UDT; and
- d) extending said annealed reverse primer or nucleic acid construct

452. (Original) The method of claim 443 or 445, wherein said primer or nucleic acid constructs are attached to a solid matrix.

453. (Original) The method of claim 452 wherein said solid matrix comprises magnetic beads, latex beads, microtitre plates and glass slides.

454. (Original) A method for synthesizing multiple copies of at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) at least one forward primer or forward nucleic acid construct wherein
    - (A) said primer or said nucleic acid construct comprises a sequence complementary to a segment of said nucleic acid target;
    - (B) said primer or said nucleic acid construct comprises a sequence for an RNA promoter;
    - (C) said primer or said nucleic acid construct comprises at least one nucleotide at the 3' end of said primer or nucleic acid construct that: a) inhibits or eliminates extension by a template independent polymerase; and b) is a substrate for extension by a template dependent polymerase;
  - (iii) template dependent reagents;
  - (iv) template independent reagents; and
  - (v) synthesizing reagents for the synthesis of a nucleic acid copy;
- b) annealing said nucleic acid target with said primer or nucleic acid construct;
- c) extending said annealed primer or said annealed nucleic acid construct by said synthesizing reagents using said nucleic acid target as a template to synthesize a copy of said nucleic acid target;
- d) using said copy as a template to form a complementary copy to render said RNA promoter sequence into double-stranded form; and
- e) carrying out a transcription reaction to provide multiple copies of said nucleic acid target.

455. (Original) The method of claim 454 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acids are complete or partial copies of nucleic acids isolated from a biological source.

456. (Original) The method of claim 455 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from said biological source.

457. (Original) The method of claim 455 wherein said nucleotides at the 3' end of said primer or nucleic acid construct comprise at least one ribonucleotide or at least one 2' nucleotide analogue at the 3' end of said primer or nucleic acid construct.

458. (Original) The method of claim 454 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
- b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
- c) synthesizing said complementary copy.

459. (Original) The method of claim 458, wherein said complementary copy is formed by a partial RNase digestion of an RNA template, hairpin formation at the 3' terminus of said nucleic acid copy or by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said nucleic acid copy.

460. (Original) The method of claim 458 further comprising:

- a) providing:
  - (i) reagents for the addition of a non-inherent UDT to said first nucleic acid copy;
  - (ii) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT;
- b) adding said non-inherent UDT to said nucleic acid copy;

- c) annealing said reverse primer or reverse nucleic acid construct to said non-inherent UDT; and
- d) extending said annealed reverse primer or nucleic acid construct

461. (Original) The method of claim 454 wherein said template dependent reagents comprise at least one of the following enzymes: E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript or any mutational variations of any of the preceding.

462. (Original) The method of claim 454 wherein said template independent reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.

463. (Original) The method of claim 460 wherein said addition of said non-inherent UDT is carried out by Terminal Deoxynucleotidyl Transferase or a ligase.

464. (Original) The method of claim 463 further comprising the step of adding a terminator nucleotide.

465. (Original) The method of claim 463 wherein said non-inherent UDT comprises a homopolymeric sequence.

466. (Original) The method of claim 460 wherein a non-inherent UDT and a terminal nucleotide are added to said nucleic acid copy by providing Terminal Deoxynucleotidyl Transferase and a mixture of terminator and non-terminator molecules.

467. (Original) The method of claim 464 or 466 wherein said terminator nucleotides comprise dideoxyribonucleotides, acyclic nucleotides, arabinosides or 3' amino nucleotides.

468. (Original) The method of claim 454 wherein said promoter sequence comprises a T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter.

469. (Original) The method of claim 454 further comprising the steps of:

- a) providing appropriate reagents; and
- b) carrying out a transcription reaction using said RNA promoter sequence.

470. (Original) The method of claim 454 further comprising the steps of:

- a) providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
- b) providing dNTPs or a mixture of dNTPs or NTPs; and
- c) creating a DNA transcript or a DNA/RNA chimeric transcript.

471. (Original) The method of claim 469 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotides thereby generating transcription products.

472. (Original) The method of claim 470 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.

473. (Original) The method of claim 469 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.

474. (Original) The method of claim 471 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a

hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

475. (Original) The method of claim 472 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

476. (Original) The method of claim 473 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

477. (Original) The method of claim 471, 472 or 473 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.

478. (Original) The method of claim 454 wherein said nucleic acid target comprises DNA or RNA.

479. (Original) A method for synthesizing multiple copies of at least one nucleic acid target comprising the steps of:

- a) providing
  - (i) at least one nucleic acid target;
  - (ii) at least one forward primer or forward nucleic acid construct and at least one reverse primer or reverse nucleic acid construct wherein
    - (A) said forward primer or said forward nucleic acid construct comprises a sequence complementary to a segment of said nucleic acid target;



- (B) said reverse primer or said reverse nucleic acid construct comprises a sequence for an RNA promoter;
- (C) said forward primer or said forward nucleic acid construct or said reverse primer or said reverse nucleic acid construct comprises at least one nucleotide at the 3' end of said forward primer or said forward nucleic acid construct or said reverse primer or reverse nucleic acid construct that: a) inhibits or eliminates extension by a template independent polymerase; and b) is a substrate for extension by a template dependent polymerase;
- (iii) template dependent reagents;
- (iv) template independent reagents; and
- (v) synthesizing reagents for the synthesis of a nucleic acid copy;
- b) annealing said nucleic acid target with said forward primer or said forward nucleic acid construct;
- c) extending said annealed forward primer or said forward nucleic acid construct by said synthesizing reagents using said nucleic acid target as a template to synthesize a copy of said nucleic acid target;
- d) annealing said copy with said reverse primer or said reverse nucleic acid construct and using said copy as a template to form a complementary copy; and
- e) carrying out a transcription reaction to produce multiple copies of said nucleic acid target.

480. (Original) The method of claim 479 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acids are complete or partial copies of nucleic acids isolated from a biological source.

481. (Original) The method of claim 480 wherein said complete or partial copies of nucleic acids are identical or complementary copies of nucleic acids isolated from said biological source.

482. (Original) The method of claim 479 wherein said nucleotide at the 3' end of said primer or nucleic acid construct comprises a ribonucleotide or a 2' nucleotide analogue at the 3' end of said primer or nucleic acid construct.

483. (Original) The method of claim 479 further comprising the steps of:

- a) providing additional synthesizing reagents for the synthesis of a complementary copy of said nucleic acid copy;
- b) separating said nucleic acid target from said first nucleic acid copy or degrading said nucleic acid target; and
- c) synthesizing said complementary copy.

484. (Original) The method of claim 483, wherein said complementary copy is formed by a partial RNase digestion of an RNA template, hairpin formation at the 3' terminus of said nucleic acid copy or by providing one or more reverse primers or reverse nucleic acid constructs complementary to sequences in said nucleic acid copy.

485. (Original) The method of claim 483 further comprising:

- a) providing:
  - (i) reagents for the addition of a non-inherent UDT to said first nucleic acid copy;
  - (ii) a reverse primer or reverse nucleic acid construct comprising sequences complementary to said UDT;
- b) adding said non-inherent UDT to said nucleic acid copy;
- c) annealing said reverse primer or reverse nucleic acid construct to said non-inherent UDT; and
- d) extending said annealed reverse primer or nucleic acid construct

486. (Original) The method of claim 479 wherein said template dependent reagents comprise at least one of the following enzymes: E. coli DNA Pol I, Klenow fragment of E. coli DNA Pol I, Bst DNA polymerase, Bca DNA polymerase, Taq DNA polymerase, Tth DNA polymerase, T4 DNA polymerase, T7 DNA polymerase, Sequenase,  $\phi$  29 DNA polymerase, ALV reverse transcriptase, MuLV reverse transcriptase, RSV reverse

transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, Sensiscript, Omniscript or any mutational variations of any of the preceding.

487. (Original) The method of claim 479 wherein said template independent reagents comprise Terminal Deoxynucleotidyl Transferase or a ligase.

488. (Original) The method of claim 479 wherein said forward primer or said forward nucleic acid construct and said reverse primer or said reverse nucleic acid construct comprise at least one nucleotide at the 3' end of said forward primer or said forward nucleic acid construct and said reverse primer or reverse nucleic acid construct that: a) inhibit or eliminate extension by a template independent polymerase; and b) are a substrate for extension by a template dependent polymerase.

489. (Original) The method of claim 479 wherein a non-inherent UDT is added to said copy after said forward primer or forward nucleic acid construct is extended, to provide a sequence complementary to said reverse primer or reverse nucleic acid construct.

490. (Original) The method of claim 489 wherein said addition of said non-inherent UDT is carried out by Terminal Deoxynucleotidyl Transferase or a ligase.

491. (Original) The method of claim 490 further comprising the step of adding a terminator nucleotide.

492. (Original) The method of claim 491 wherein said non-inherent UDT comprises a homopolymeric sequence.

493. (Original) The method of claim 489 wherein a non-inherent UDT and a terminal nucleotide are added to said nucleic acid copy by providing Terminal Deoxynucleotidyl Transferase and a mixture of terminator and non-terminator molecules.

494. (Original) The method of claim 491 or 493 wherein said terminator nucleotides comprise dideoxynucleotides, acyclic nucleotides, arabinosides or 3' amino nucleotides.
495. (Original) The method of claim 479 wherein said promoter sequence comprises a T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter.
496. (Original) The method of claim 479 further comprising the steps of:
- providing appropriate reagents; and
  - carrying out a transcription reaction using said RNA promoter sequence.
497. (Original) The method of claim 479 further comprising the steps of:
- providing reagents for RNA transcription, said reagents comprising  $Mn^{++}$ , mutated RNA polymerases or a combination thereof;
  - providing dNTPs or a mixture of dNTPs or NTPs; and
  - creating a DNA transcript or a DNA/RNA chimeric transcript.
498. (Original) The method of claim 496 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotides thereby generating transcription products.
499. (Original) The method of claim 497 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
500. (Original) The method of claim 496 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.
501. (Original) The method of claim 498 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a

chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

502. (Original) The method of claim 499 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

503. (Original) The method of claim 500 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

504. (Original) The method of claim 498, 499 or 500 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.

505. (Original) The method of claim 479 wherein said nucleic acid target comprises DNA or RNA.

506. (Original) A method of synthesizing a double-stranded DNA copy from at least one RNA target comprising the steps of:

- a) providing:
  - (i) at least one RNA target;
  - (ii) at least one forward primer or forward nucleic acid construct complementary to a sequence in said RNA target wherein said forward primer

or forward nucleic acid construct comprises at least one ribonucleotide or nucleotide analogue at its 3' end;

- (iii) synthesizing reagents for template dependent synthesis of a first cDNA copy by the extension of said forward primer or forward nucleic acid construct;
  - (iv) addition reagents for the non-template directed addition of a non-inherent UDT to the 3' end of said first cDNA copy wherein said non-inherent UDT comprises a primer binding site for second strand synthesis and wherein said addition reagents add said non-inherent UDT more efficiently to said first cDNA copy than to unextended forward primers or forward nucleic acid constructs;
  - (v) at least one reverse primer or reverse nucleic acid construct comprising sequences complementary to said primer binding site in said non-inherent UDT;
  - (vi) a second set of synthesizing reagents for second strand synthesis; and
- b) annealing said RNA target with said forward primer or forward nucleic acid construct;
- c) extending said annealed forward primer or forward nucleic acid construct using said RNA target as a template to form at least one first cDNA copy;
  - d) adding a non-inherent UDT to the 3' end of said first cDNA copy using said addition reagents to form at least one extended first cDNA copy;
  - e) annealing said extended first cDNA copy with said reverse primer; and
  - f) extending said annealed reverse primer or reverse nucleic acid construct using said extended first cDNA copy as a template and said second set of synthesizing reagents to synthesize a double-stranded copy of said RNA target.

507. (Original) The method of claim 506 wherein said forward primer or forward nucleic acid construct or said reverse primer or reverse nucleic acid construct comprises an RNA promoter.

508. (Original) The method of claim 507 wherein said promoter sequence comprises T3 RNA promoter, T7 RNA promoter or SP6 RNA promoter sequences.
509. (Original) The method of claim 507 further comprising the steps of:
- a) providing appropriate reagents;
  - b) carrying out a transcription reaction using said promoter sequence.
510. (Original) The method of claim 507 comprising the steps of:
- a) providing reagents for RNA transcription, said reagents comprising Mg<sup>++</sup>, mutated RNA polymerases or a combination thereof;
  - b) providing dNTPs or a mixture of dNTPs and NTPS; and
  - c) creating a DNA transcript or a DNA/RNA chimeric transcript.
511. (Original) The method of claim 509 wherein said transcription reaction is carried out in the presence of at least one labeled nucleotide, thereby generating labeled transcription products.
512. (Original) The method of claim 510 wherein said DNA transcript or DNA/RNA chimeric transcript is carried out in the presence of at least one labeled nucleotide, thereby generating labeled DNA or labeled DNA/RNA chimeric transcription products.
513. (Original) The method of claim 509 further comprising the step of synthesizing cDNA in the presence of at least one labeled nucleotide, thereby generating labeled cDNA products.
514. (Original) The method of claim 511 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a

hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

515. (Original) The method of claim 512 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

516. (Original) The method of claim 513 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

517. (Original) The method of claim 511, 512 or 513 wherein said labeled products are used as templates for at least one additional round of nucleic acid synthesis.

518. (Original) A method for the amplification of a library of nucleic acids comprising the steps of:

- a) providing:
  - (i) nucleic acids to be amplified
  - (ii) at least one first primer comprising two segments wherein the first segment comprises a first UDT and the second segment comprises an RNA promoter sequence;
  - (iii) at least one second primer comprising a second UDT;
  - (iv) reagents for amplifying procedures, wherein said amplifying procedures comprise TMA, NASBA or 3SR; and
- b) carrying out said amplifying procedures to amplify said library of nucleic acids.



519. (Original) The method of claim 518 wherein said nucleic acids comprise single-stranded nucleic acids.

520 (Original) The method of claim 518 wherein said nucleic acids comprise double-stranded nucleic acids that have been rendered single-stranded.

521 (Original) The method of claim 518 wherein said library of nucleic acid targets are isolated from a biological source or said library of nucleic acid targets are complete or partial copies of nucleic acids isolated from a biological source.

522. (Original) The method of claim 521 wherein said complete or partial copies of nucleic acids are identical or complementary copies of said nucleic acids isolated from a biological source.

523. (Original) The method of claim 521 further comprising the step of adding a non-inherent UDT to said nucleic acids isolated from a biological source.

524. (Original) The method of claim 522 further comprising the step of adding a non-inherent UDT to said nucleic acids isolated from a biological source.

525 (Original) The method of claim 523 wherein said first UDT and said second UDT comprise the same sequences.

526. (Original) The method of claim 524 wherein said first UDT and said second UDT comprise the same sequences.

527. (Original) The method of claim 523 wherein said first UDT and said second UDT comprise different sequences.

528. (Original) The method of claim 524 wherein said first UDT and said second UDT comprise different sequences.

529. (Original) The method of claim 525 or 526 wherein said first UDT and said second UDT are inherent UDTs.

530. (Original) The method of claim 527 or 528 wherein said first UDT is an inherent UDT and said second UDT is a non-inherent UDT.

531. (Original) The method of claims 527 or 528 wherein said first UDT is a non-inherent UDT and said second UDT is an inherent UDT.

532. (Original) The method of claim 525 or 526 wherein said first UDT and said second UDT are non-inherent UDTs.

533 (Original) The method of claim 529, 530, 531 or 532 wherein said inherent UDTs comprise a poly A segment or a consensus segment.

534. (Original) The method of claim 533 wherein said consensus segments comprise signal sites for poly A addition, splicing elements, and multicopy repeats.

535. (Original) The method of claim 529, 530, 531 or 532 wherein said non-inherent UDTs comprise a homopolymeric sequence.

536. (Original) The method of claim 518 wherein said nucleic acids comprise a label.

537. (Original) The method of claim 536 wherein said labeled nucleotides comprise a fluorescent compound, a phosphorescent compound, a chemiluminescent compound, a chelating compound, an electron dense compound, a magnetic compound, an intercalating compound, an energy transfer compound, an antibody, an antigen, a

hapten, a receptor, a hormone, a ligand, an enzyme, or any combination of the preceding.

538. (Original) A composition of matter comprising a set of nucleic acid constructs wherein said nucleic acid constructs each comprise a first portion and a second portion, wherein said first portion comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second portion comprises a single-stranded tail comprising a G, A, T or C nucleotide at the terminus of said tail.

539. (Original) The composition of claim 538 wherein said second portion of said set of nucleic acid primers or nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of said tail.

540. (Original) The composition of claims 538 and 539 wherein said nucleic acid primers or nucleic acid constructs comprise an RNA promoter sequence.

541. (Original) The composition of claim 540 wherein said RNA promoter sequence comprises a T3 RNA promoter sequence, T7 RNA promoter sequence or SP6 RNA promoter sequence.

542. (Original) The composition of claim 538 or 539 wherein said single-stranded tail comprises a 3' tail.

543. (Original) The composition of claim 538 or 539 wherein said single-stranded tail comprises a 5' tail.

544. (Original) A composition of matter comprising a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is

a single-stranded segment of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T.

545. (Original) A composition of matter comprising a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a single-stranded segment wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T.

546. (Original) The composition of claims 544 or 545 wherein said nucleic acid constructs comprise an RNA promoter sequence.

547. (Original) The composition of claim 546 wherein said RNA polymerase promoter sequence comprises a T3 RNA promoter sequence, T7 RNA promoter sequence or SP6 RNA promoter sequence.

548. (Original) The composition of claim 544 or 545 wherein said single-stranded segment comprises a 3' tail.

549. (Original) The composition of claim 544 or 545 wherein said single-stranded segment comprises a 5' tail.

550. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:

- a) providing
  - (i) a collection of single stranded target nucleic acids;
  - (ii) a set of nucleic acid constructs comprising:
    - (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said

discrete sequence common to all members of said set, and said second segment comprises a single-stranded tail comprising G, A, T or C at the terminus of said tail;

(B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

(iii) reagents for nucleic acid ligation;

- b) forming a mixture of said target nucleic acids, said set of nucleic acid constructs and said reagents; and
- c) ligating said set of nucleic acid constructs to the ends of said target nucleic acids.

551. (Original) The method of claim 550 wherein said single stranded target nucleic acid comprises RNA.

552. (Original) The method of claim 550 wherein said single stranded target nucleic acid comprises DNA.

553. (Original) The method of claim 550 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.
554. (Original) The method of claim 553 wherein said set of nucleic acid constructs comprise 3' single stranded tails and ligation occurs at the 3' ends of said target nucleic acids.
555. (Original) The method of claim 553 wherein said set of nucleic acid constructs comprise 5' single stranded tails and ligation occurs at the 5' ends of said target nucleic acids.
556. (Original) The method of claim 554 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.
557. (Original) The method of claim 554 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.
558. (Original) The method of claim 555 further comprising:
- a) providing:
    - (i) Terminal Deoxynucleotidyl Transferase or poly A polymerase;
    - (ii) primers or nucleic acid constructs;
    - (iii) reagents for the addition of a non-inherent UDT; and
    - (iv) reagents for the extension of primers or nucleic acid constructs;
  - b) adding a non-inherent UDT to the 3' end of said target nucleic acids;
  - c) annealing said primers or nucleic acid constructs to said non-inherent UDTs; and

d) extending said annealed primers or nucleic acid constructs to form complementary copies of said target nucleic acids.

559. (Original) The method of claim 556, 557 or 558 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.

560. (Original) The method of claim 559 further comprising the steps of:

- a) providing transcription reagents; and
- b) carrying out transcription.

561. (Original) The method of claim 553, 554, 555, 556, 557 or 558 further comprising the steps of:

- a) providing Polymerase Chain Reaction reagents; and
- b) carrying out Polymerase Chain Reaction.

562. (Original) The method of claim 553, 554, 555, 556, 557 or 558 further comprising the steps of:

- a) providing isothermal amplification reagents; and
- b) carrying out isothermal amplification.

563. (Original) The method of claim 562 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.

564. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:

- a) providing:
  - (i) a collection of single stranded target nucleic acids;
  - (ii) a set of nucleic acid constructs comprising:
    - (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said

discrete sequence common to all members of said set, and said second segment comprises a single-stranded tail comprising G, A, T or C at the terminus of said tail;

(B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

(iii) reagents for nucleic acid ligation;

- b) dividing said collection of target nucleic acids into a first portion and a second portion;
- c) dividing said set of nucleic acid constructs into a first subset and a second subset, wherein the members of said first subset are different from the members of said second subset;
- d) mixing said first portion with said first subset of said nucleic acid constructs and said reagents;
- e) ligating said first portion to said first subset of said nucleic acid constructs to produce a first group;
- f) removing unligated nucleic acid constructs from said first group;



- g) mixing said second portion with said second subset of nucleic acid constructs;
- h) ligating said second portion with said second subset of nucleic acid constructs to produce a second group;
- i) removing unligated nucleic acid constructs from said second group; and
- j) mixing said first group and said second group to form a collection of target nucleic acids with added nucleic acid sequences.

565. (Original) The method of claim 564 wherein said single-stranded target nucleic acids comprise RNA.

566. (Original) The method of claim 564 wherein said single-stranded target nucleic acids comprise DNA.

567. (Original) The method of claim 564 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.

568. (Original) The method of claim 567 wherein said set of nucleic acid constructs comprise 3' single stranded tails and ligation occurs at the 3' ends of said target nucleic acids.

569. (Original) The method of claim 567 wherein said set of nucleic acid constructs comprise 5' single stranded tails and ligation occurs at the 5' ends of said target nucleic acids.

570. (Original) The method of claim 568 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.

571. (Original) The method of claim 568 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct

comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.

572. (Original) The method of claim 569 further comprising:
- a) providing:
    - (i) Terminal Deoxynucleotidyl Transferase or poly A polymerase;
    - (ii) primers or nucleic acid constructs;
    - (v) reagents for the addition of a non-inherent UDT; and
    - (vi) reagents for the extension of primers or nucleic acid constructs;
  - b) adding a non-inherent UDT to the 3' end of said target nucleic acids;
  - c) annealing said primers or nucleic acid constructs to said non-inherent UDTs; and
  - d) extending said annealed primers or nucleic acid constructs to form complementary copies of said target nucleic acids.
573. (Original) The method of claim 570, 571 or 572 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.
574. (Original) The method of claim 573 further comprising the steps of:
- a) providing transcription reagents; and
  - b) carrying out transcription.
575. (Original) The method of claim 567, 568, 569, 570, 571 or 572 further comprising the steps of:
- a) providing Polymerase Chain Reaction reagents; and
  - b) carrying out Polymerase Chain Reaction.
576. (Original) The method of claim 567, 568, 569, 570, 571 or 572 further comprising the steps of:
- a) providing isothermal amplification reagents; and
  - b) carrying out isothermal amplification.

577. (Original) The method of claim 576 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.
578. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:
- a) providing:
- (i) a collection of single stranded target nucleic acids;
  - (ii) a set of nucleic acid constructs comprising:
    - (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a single-stranded tail comprising G, A, T or C at the terminus of said tail;
    - (B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a single-stranded tail;
    - (C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or
    - (D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a single-stranded segment wherein  $N_1$  is a penultimate

nucleotide comprising A, G, C or T and N<sub>2</sub> is a terminal nucleotide comprising A, G, C or T;

- (iii) reagents for nucleic acid ligation;
- b) dividing said set of nucleic acid constructs into a first and second subset wherein the members of said first subset are different from the members of said second subset;
- c) mixing said collection of target nucleic acids with said first subset and said reagents;
- d) ligating said target nucleic acids with said first subset to form a first group;
- e) removing unligated nucleic acid constructs from said first group;
- f) mixing said first group with said second subset and said reagents; and
- g) ligating said first group to said second subset to form a collection of target nucleic acids with added nucleic acid sequences.

579. (Original) The method of claim 578 wherein said single-stranded target nucleic acids comprise RNA.

580. (Original) The method of claim 578 wherein said single-stranded target nucleic acids comprise DNA.

581. (Original) The method of claim 578 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.

582. (Original) The method of claim 581 wherein said set of nucleic acid constructs comprise 3' single stranded tails and ligation occurs at the 3' ends of said target nucleic acids.

583. (Original) The method of claim 581 wherein said set of nucleic acid constructs comprise 5' single stranded tails and ligation occurs at the 5' ends of said target nucleic acids.

584. (Original) The method of claim 582 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.
585. (Original) The method of claim 582 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.
586. (Original) The method of claim 583 further comprising:
- a) providing:
    - (i) Terminal Deoxynucleotidyl Transferase or poly A polymerase;
    - (ii) primers or nucleic acid constructs;
    - (vii) reagents for the addition of a non-inherent UDT; and
    - (viii) reagents for the extension of primers or nucleic acid constructs;
  - b) adding a non-inherent UDT to the 3' end of said target nucleic acids;
  - c) annealing said primers or nucleic acid constructs to said non-inherent UDTs; and
  - d) extending said annealed primers or nucleic acid constructs to form complementary copies of said target nucleic acids.
587. (Original) The method of claim 584, 585 or 586 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.
588. (Original) The method of claim 587 further comprising the steps of:
- a) providing transcription reagents; and
  - b) carrying out transcription.
589. (Original) The method of claim 581, 582, 583, 584, 585 or 586 further comprising the steps of:
- a) providing Polymerase Chain Reaction reagents; and

b) carrying out Polymerase Chain Reaction.

590. (Original) The method of claim 581, 582, 583, 584, 585 or 586 further comprising the steps of:

- a) providing isothermal amplification reagents; and
- b) carrying out isothermal amplification.

591. (Original) The method of claim 590 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.

592. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:

- a) providing:
  - (i) a collection of single stranded target nucleic acids;
  - (ii) a first set of nucleic acid constructs with 3' single-stranded tails, said first set comprising:

- (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 3' single-stranded tail comprising G, A, T or C at the terminus of said tail;

- (B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 3' single-stranded tail;

- (C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a

discrete sequence of at least 10 nucleotides and  $N_y$  is a 3' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 3' single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

(iii) a second set of nucleic acid constructs with 5' single-stranded tails, said second set comprising:

(A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 5' single-stranded tail comprising G, A, T or C at the terminus of said tail;

(B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 5' single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a 5' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 5' single-stranded tail wherein  $N_1$  is a penultimate

nucleotide comprising A, G, C or T and N<sub>2</sub> is a terminal nucleotide comprising A, G, C or T;

- (iv) reagents for nucleic acid ligation;
- b) forming a mixture of said target nucleic acids, said first set of nucleic acid constructs, said second set of nucleic acid constructs and said reagents; and
- c) ligating said first set of nucleic acid constructs to the 3' ends of said target nucleic acids and said second set of nucleic acid constructs to the 5' ends of said target nucleic acids.

593. (Original) The method of claim 592 wherein said single-stranded target nucleic acids comprise RNA.

594. (Original) The method of claim 592 wherein said single-stranded target nucleic acids comprise DNA.

595. (Original) The method of claim 592 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.

596. (Original) The method of claim 592 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.

597. (Original) The method of claim 592 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.

598. (Original) The method of claim 596 or 597 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.



599. (Original) The method of claim 598 further comprising the steps of:
- a) providing transcription reagents; and
  - b) carrying out transcription.
600. (Original) The method of claim 595 further comprising the steps of:
- a) providing Polymerase Chain Reaction reagents; and
  - b) carrying out Polymerase Chain Reaction.
601. (Original) The method of claim 595 further comprising the steps of:
- a) providing isothermal amplification reagents; and
  - b) carrying out isothermal amplification.
602. (Original) The method of claim 601 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.
603. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:
- a) providing:
    - (i) a collection of single stranded target nucleic acids;
    - (ii) a first set of nucleic acid constructs with 3' single-stranded tails, said first set comprising:
      - (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 3' single-stranded tail comprising G, A, T or C at the terminus of said tail;
      - (B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said

discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 3' single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a 3' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 3' single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

(iii) a second set of nucleic acid constructs with 5' single-stranded tails, said second set comprising:

(A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 5' single-stranded tail comprising G, A, T or C at the terminus of said tail;

(B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 5' single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a

discrete sequence of at least 10 nucleotides and  $N_y$  is a 5' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 5' single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

- (iv) reagents for nucleic acid ligation;
- b) dividing said collection of target nucleic acids into a first portion and a second portion;
- c) dividing said first set of nucleic acid constructs into a first subset and a second subset, wherein the members of said first subset are different from the members of said second subset;
- d) dividing said second set of nucleic acid constructs into a third subset and a fourth subset, wherein the members of said third subset are different from the members of said fourth subset;
- e) mixing said first portion with said first subset, said third subset and said reagents;
- f) ligating said first subset to the 3' ends and said third subset to the 5' ends of said first portion of nucleic acid targets to form a first group;
- g) removing unligated nucleic acid constructs from said first group;
- h) mixing said second portion with said second subset, said fourth subset and said reagents;
- i) ligating said second subset to the 3' ends and said fourth subset to the 5' ends of said second portion of nucleic acid targets to form a second group;
- j) removing unligated nucleic acid constructs from said second group;
- k) mixing said first group and said second group to form a collection of nucleic acid constructs with nucleic acid sequences added to their 3' and 5' ends.

604. (Original) The method of claim 603 wherein said single-stranded target nucleic acids comprise RNA.
605. (Original) The method of claim 603 wherein said single-stranded target nucleic acids comprise DNA.
606. (Original) The method of claim 603 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.
607. (Original) The method of claim 603 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.
608. (Original) The method of claim 603 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.
609. (Original) The method of claim 607 or 608 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.
610. (Original) The method of claim 609 further comprising the steps of:
- a) providing transcription reagents; and
  - b) carrying out transcription.
611. (Original) The method of 606 claim further comprising the steps of:
- a) providing Polymerase Chain Reaction reagents; and
  - b) carrying out Polymerase Chain Reaction.

612. (Original) The method of claim 606 further comprising the steps of:
- a) providing isothermal amplification reagents; and
  - b) carrying out isothermal amplification.
613. (Original) The method of claim 612 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.
614. (Original) A method for adding nucleic acid sequences to a collection of target nucleic acids comprising the steps of:
- a) providing:
    - (i) a collection of single stranded target nucleic acids;
    - (ii) a first set of nucleic acid constructs with 3' single-stranded tails, said first set comprising:
      - (A) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 3' single-stranded tail comprising G, A, T or C at the terminus of said tail;
      - (B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 3' single-stranded tail;
      - (C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a 3' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 3' single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

(iii) a second set of nucleic acid constructs with 5' single-stranded tails, said second set comprising:

(A) a set wherein said -nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment comprises a 5' single-stranded tail comprising G, A, T or C at the terminus of said tail;

(B) a set wherein said nucleic acid constructs each comprise a first segment and a second segment, wherein said first segment comprises a double-stranded segment with a discrete sequence of at least 10 nucleotides, said discrete sequence common to all members of said set, and said second segment of said set of nucleic acid constructs comprises a permutational set wherein each member of said permutational set comprises GA, GC, GT, GG, AA, AC, AT, AG, TA, TC, TT, TG, CA, CC, CT or CG at the terminus of a 5' single-stranded tail;

(C) a set of nucleic acid constructs having the formula  $Q_x-N_y$ , wherein  $Q_x$  is a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides and  $N_y$  is a 5' single-stranded tail of at least one nucleotide wherein the terminal nucleotide comprises G, A, C or T; or

(D) a permutational set of nucleic acid constructs having the formula  $Q_z-N_1N_2$  wherein  $Q_z$  comprises a partially or completely double-stranded nucleic acid segment comprising a discrete sequence of at least 10 nucleotides,  $N_1N_2$  comprises all or part of a 5' single-stranded tail wherein  $N_1$  is a penultimate nucleotide comprising A, G, C or T and  $N_2$  is a terminal nucleotide comprising A, G, C or T;

- (iv) reagents for nucleic acid ligation;
- a) dividing said first set of nucleic acid constructs into a first subset and a second subset, wherein the members of said first subset are different from the members of said second subset;
- b) dividing said second set of nucleic acid constructs into a third subset and a fourth subset, wherein the members of said third subset are different from the members of said fourth subset;
- c) mixing said collection of target nucleic acids with said first subset, said third subset and said reagents;
- d) ligating said first subset to the 3' ends and said third subset to the 5' ends of said nucleic acid targets;
- e) removing unligated nucleic acid constructs;
- f) mixing said nucleic acid targets with said second subset, said fourth subset and said reagents; and
- g) ligating said second subset to the 3' ends and said fourth subset to the 5' ends of said nucleic acid targets to form a collection of nucleic acids with nucleic acid sequences added to the 3' and 5' ends.

615. (Original) The method of claim 614 wherein said single-stranded target nucleic acids comprise RNA.

616. (Original) The method of claim 614 wherein said single-stranded target nucleic acids comprise DNA.

617. (Original) The method of claim 614 wherein said discrete sequence comprises a primer binding site, an RNA promoter sequence, a capture sequence, a homopolymeric sequence, or a combination thereof.

618. (Original) The method of claim 614 further comprising the step of providing appropriate reagents and extending the 3' end of said nucleic acid constructs to form complementary copies of said target nucleic acids.

619. (Original) The method of claim 614 further comprising the step of providing appropriate reagents and providing a primer or nucleic acid construct comprising sequences complementary to said primer binding site, annealing said primer or nucleic acid construct to thereby form complementary copies of said target nucleic acids.
620. (Original) The method of claim 618 or 619 wherein said discrete sequence or said primers or nucleic acid constructs comprise an RNA promoter sequence.
621. (Original) The method of claim 587 further comprising the steps of:
- a) providing transcription reagents; and
  - b) carrying out transcription.
622. (Original) The method of claim 617 further comprising the steps of:
- a) providing Polymerase Chain Reaction reagents; and
  - b) carrying out Polymerase Chain Reaction.
623. (Original) The method of claim 617 further comprising the steps of:
- a) providing isothermal amplification reagents; and
  - b) carrying out isothermal amplification.
624. (Original) The method of claim 623 wherein said isothermal amplification comprises TMA, 3SR, NASBA, LIDA, or Hairpin mediated amplification.
625. (New) A method for synthesizing a copy of at least one nucleic acid target comprising the steps of:
- a) providing:
    - (i) at least one nucleic acid target;
    - (ii) at least one primer or nucleic acid construct complementary to a sequence in said nucleic acid target, wherein said primer or nucleic acid construct



comprise one or more terminal nucleotides at their 3' ends, wherein said terminal nucleotides comprise nucleotide analogues with substitutions on the 2' position of the ribose ring; and

(iii) template dependent reagents for the synthesis of a nucleic acid copy;

and

b) annealing said primer or nucleic acid construct to said nucleic acid target; and

c) extending said primer or nucleic acid construct by said synthesizing reagents using at least one nucleic acid target as a template to synthesize a copy of said nucleic acid target.

626. (New) The method of claim 506, wherein said forward primer or forward nucleic acid construct or said reverse primer or reverse nucleic acid construct comprises at the 3' end at least one ribonucleotide or a nucleotide analogue with a 2' modification .